Epidemiology of *Potato virus S* and *Potato virus X* in seed potato in Tasmania, Australia

by

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Authority of Access

This thesis contains no material which has been accepted for the award of any other higher degree of diploma in any University, and to the best of my knowledge contains no paraphrase of material written or published by any other person except where due reference is made in the text of this thesis.

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Abstract

Potato virus S (PVS) and *Potato virus X* (PVX) are common viruses of potato (*Solanum tuberosum* L.) and are transmitted by plant-to-plant contact. Some PVS strains are aphid transmissible. Both viruses can reportedly cause up to 10-15% yield loss in potato crops. This project investigated the epidemiology of PVS and PVX in Tasmanian seed potatoes and their effect on yield.

Surveys for PVS and PVX were undertaken in seed potato crops in 2002/2003 and 2003/2004. During 2002/2003, PVS and PVX were detected in 66.7% of 225 crops and 12.9% of 232 crops respectively, with a mean incidence of 17.9% and 0.3% respectively. In 107 seed crops surveyed during 2003/2004, PVS and PVX occurred in 42.1% and 4.7% crops respectively with a mean incidence of 8.1% and 1.0% respectively. PVS was more prevalent and occurred at greater incidence in the north-east of Tasmania, while PVX was restricted to the north-west.

Three years of field trials with cv. Russet Burbank showed a significant ($P<0.001$) negative linear relationship between incidence of PVS and processing yield. Regression analysis predicted reductions in processing yield of 5.6, 6.3 and 10.1 t/ha over three years as a result of complete infection with PVS.

Virus spread was assessed by regular sampling during the growing season in four commercial fields of seed potatoes cv. Russet Burbank. PVS incidence did not increase in two fields, but increased by 5.2% between 31 and 107 days after planting (DAP), and 25.5% between 30 and 105 DAP in each of the other fields. Known aphid

vectors of PVS were not detected on sticky traps at any field. PVX incidence increased by 10.1% between 31 and 107 DAP in one field. The other fields were free of detectable PVX early in the season, but one had trace infection (0.1%) at 129 DAP. Spatial analysis detected aggregation of PVS infected plants early in the season in two fields, suggesting virus transmission occurred during planting or seed-cutting. Overseas studies suggest both viruses are readily transmitted by seed cutting, but several Tasmanian studies show limited seed-cutting transmission of PVS. Increased PVS incidence late in the season in one field was associated with aggregation of PVS infected plants along, but not across rows, suggesting mechanical transmission of the virus. PVS was detected infrequently, and PVX was not detected, in weeds from four potato fields, suggesting weeds were not a major source of inoculum

Fifty-two isolates of PVS were characterised as PVS^{O} and three isolates as PVS^{A} based on symptom expression and capacity for systemic invasion of indicator host *Chenopodium quinoa*. Subsequent analysis of 21 PVS isolates by RT-PCR-RFLP, including isolates identified as PVS^{A}-like on *C. quinoa*, demonstrated RFLP patterns predicted for PVS^{O}. Results suggest that the differentiation of PVS into two strains may need revision, and a need for a more in-depth study of the phylogeny and biological properties of PVS isolates from a wider geographic area, to better understand strain relationships. Thirteen PVS isolates latently infected *Solanum laciniatum* Ait., following mechanical inoculation. This is the first record of *S. laciniatum* as a host of PVS.

1. Introduction

Potato (*Solanum tuberosum* L.) is an important vegetable crop grown in Tasmania, accounting for over 75% of the total vegetable production in 2004 (Anon. 2006a). Approximately 25% of Australian potato production occurs in Tasmania. In 2004/2005 the Tasmanian potato industry included 449 growers, with a total of 7 350 hectares, with a farm gate value of A$89 million (Anon. 2006a).

Historically the potato industry represents the largest sector of the vegetable industry in Tasmania. Although traditionally the potato industry has been centered around the northwest coast a trend has developed with potatoes being grown on sandy soils around the east coast and midlands (Kirkwood 2003a). The Tasmanian potato industry comprises three sectors including the seed potato industry, fresh potato industry and processing potato industry, with the processing sector comprising 80% of the industry with the variety Russet Burbank predominating (Kirkwood 2003a). Seed potato production predominately occurs along the northwest region of Tasmania with approximately 90 seed growers in 2005 (L. White, Department of Primary Industry and Water (DPIW), *personal communication*). The seed certification program was introduced into Tasmania in the 1930s, with the aims of minimising disease and improving yield (Taylor 2003). In recent years seed certification in Australia has been regulated under the National Standard for Certification of Seed Potato. During production of seed potatoes, tubers are increased over a maximum of four growing seasons prior to release for use as commercial crops. Each growing season represents one generation (G) of seed potato. Crops are visually

inspected by seed certification officers twice during the growing season for diseases, including symptoms of virus. For many years, the Tasmanian seed potato industry has been considered relatively virus free through adherence to the National Standard and through natural geographical barriers. Sporadic occurrences of *Potato virus S* (Family Flexiviridae, genus Carlavirus, PVS), *Potato virus X* (Family Flexiviridae, genus Potexvirus, PVX), *Potato leafroll virus* (Family Luteoviridae, genus Polerovirus, PLRV) and *Tomato spotted wilt virus* (Family Bunyaviridae, genus Tospovirus, TSWV) have been reported. However, a limited survey conducted by DPIW in 2001 identified PVS and to a lesser extent PVX as being prevalent in some Tasmanian seed stocks (Kirkwood 2003b).

PVS and PVX are widespread in other countries and some states of Australia. The widespread nature of these viruses is of concern to the industry due to their ease of spread and because little is known of their impact on yield or the rate of spread of these viruses in Australia. In other countries, PVS and PVX has been associated with yield losses ranging from 0-20% depending on virus strain, potato variety and environmental conditions (Banttari *et al.* 1993). Significant yield loss (up to 40%) has also been reported in potato crops possessing co-infections of PVS and PVX (Stevenson *et al.* 2001). At present, no studies have been conducted on the effect of PVS on local varieties in Australia.

Two strains of PVS are currently recognised, the ordinary (PVS^O) and the Andean strains (PVS^A) (Brunt 2001a). Research in other countries has shown that PVS is transmitted

readily through mechanical contact between infected and healthy plant material. In addition some strains of PVS are also transmissible inefficiently in a non-persistent manner by some aphid species. However, basic information about PVS in Tasmania is lacking. It had not been determined which strain(s) of PVS are present in Tasmania. PVX strains are characterised into several groupings based on the presence of genes for the hypersensitive reaction in potato (Cockerham 1955). In Australia Group 1 and 3 PVX strain are reported to be widespread. The implementation of management strategies is reliant on the understanding of epidemiological knowledge of mechanisms of disease spread, impact of yield and the strains present in Tasmania. Information on the epidemiology of PVS and PVX in Tasmania would assist the Tasmanian potato industry.

This project undertook a study of the Tasmanian seed potato scheme with the aims of:

1. Identifying the prevalence and incidence of PVS and PVX.
2. Characterising the strain(s) of PVS present.
3. Determining mechanisms of spread of PVS and PVX.
4. Investigating the effect of local strains of PVS and PVX on yield of the processing variety Russet Burbank grown under local conditions.
5. Assessing spatial patterns of PVS and PVX in seed potato.

2. Literature Review

2.1 Potatoes

2.1.1 Introduction

Potato (*Solanum tuberosum* L.) originated from South America in the Andean highlands and globally is ranked as the fourth most important food crop (Rowe 1993). With the exception of Antarctica, potato is grown on all continents (Rowe and Powelson 2002). The main potato producing countries in order of importance are China, the Russia Federation, the United States and Poland (FAO 2007) (Table 2.1).

In addition to providing a primary food source for many nations, the importance of potato extends to use of potato starch for production of goods such as adhesives, paper and textiles. Potato starch is also used as a low-fat food additive and also in edible binding agents. In addition disposable diapers are made from among other things highly absorbent biodegradable material produced from potato. Other products from potato include starch used to assist the smooth running of oil well drilling components and binding ingredients for cosmetic creams and lipsticks. In addition potato starch products provide an alternative source to the use of petroleum based chemicals such as a flocculation agent in water purification systems (Rowe and Powelson 2002; Hughes 1991).

Table 2.1. Statistical data of regions and typical countries (from Struik and Wiersema 1999, adapted from International Potato Centre (CIP) 1998).

	Projected population in year 2000 (millions)	**Agricultural share of Gross Domestic Product (%)**	**Rank of importance of potato vs. other crops**	**Area (000 ha)**	**Production (000 t)**	**Yield (t/ha)**
Asia						
China	1,225	20	5	3,489	47,777	14
India	1,022	27	3	1,116	18,627	17
Iran	68	-	2	152	3,182	21
Bangladesh	120	30	2	133	1,489	11
Africa						
Egypt	62	16	5	130	2,656	20
South Africa	46	5	3	56	1,539	28
Algeria	31	12	2	80	1,099	14
Malawi	12	36	2	51	379	7
Latin America						
Columbia	40	16	2	170	2,770	16
Brazil	175	14	7	182	2,701	15
Peru	26	7	1	240	2,355	10
Argentina	37	6	4	98	2,155	22
North America						
USA	275	-	4	556	21,200	38
Europe						
Poland	39	6	1	1,390	24,295	17
Germany	82	1	3	354	12,530	35
Netherlands	16	3	1	183	7,834	43
Eurasia						
Russian Federation	147	7	1	3,389	38,534	11
WORLD	6,123	-	4	18,381	295,118	16

Agricultural share of Gross Domestic Product is based on 1997 data. Ranking of crop's importance is based on fresh weight. Production and yield are based on fresh weight and are averages over the period 1995-1997. A dash means no reliable data available. Source: Struik and Wiersema (1999).

2.1.2 Potato biology and physiology

The potato (*Solanum tuberosum* L.) is an annual dicotyledonous herbaceous plant endemic to South America (Stevenson *et al.* 2001). The potato belongs to the *Solanaceae* family along with other commercially important crops such as tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum*), eggplant (*S. megongena*) and pepper (*Capsicum annuum*), and other crops of lesser economic importance such as Pepino dulce (*S. muricatum*) (Rabinowitch and Levy 2001).

Reproduction of potato may occur asexually by tuber formation at the terminus of stolons. In addition, flowers produced by the plant develop into poisonous berries, purplish-green to green in colour. Berries range in diameter from between 10 and 20 mm. Traditionally, breeding programs have utilised true potato seeds (TPS). Tuber formation in potato plants is promoted by environmental factors such as low temperature and a short photoperiod (Rabinowitch and Levy 2001).

2.1.3 Importance of viruses of potato

Shortly after the introduction of potato to Europe in 1570, "running out" or "degeneration" was reported in potato crops. The practise of continuous vegetative propagation was attributed to physiological reasons (Salaman 1970; Salazar 2003). However, in the 19th century diseases were determined to cause degeneration of potato, in particular diseases later shown to be caused by viruses (Salazar 2003). At least 37 viruses are known to infect potato (Brunt 2001a), many of which are believed

to have originated from South America in the Andean region (Jones 1983) (Table 2.2.).

Table 2.2. Plant virus genera or families having one or more members that affect potatoes (from Salazar 2003).

Virus genus or family	Member virus (es)*	Particle size (nm)	Means of natural transmission	Geographic distribution
Alfamovirus	AMV, PYV	58+52+42 x 18	Aphids	Worldwide
Bunyaviridae	ToSWV	80 (envelope)	Thrips	S.America, S.E.Asia
Carlavirus	PVS, PVM, PVP, PRDV	640 X 11	Mechanical, aphids Mechanical, aphids?	Worldwide S. America
Comovirus	APMoV	28 (diam.)	Mechanical, beetles?	Andes
Crinivirus	PYVV	c. 720	White flies	South America
Cucumovirus	CMV	30 (diam.)	Aphids	Europe
Geminiviridae	BCTV, PYMV SALCV	17 (pairs) 17 (triplets)	Leafhopper Unknown	South America Peru
Ilarvirus	TSV	28 (diam.)	Thrips	Brazil, Peru
Luteovirus	PLRV, SYV	25 (diam.)	Aphids	Worldwide
Necrovirus	TNV	26 (diam.)	Fungus	Europe, Andes
Nepovirus	PBRV, TRSV ToBRV, AVB(O) PVU	28 (diam.)	Nematodes	Europe, USA
Potexvirus	PVX, PAMV, PapMV, PepMV	520 x 13	Mechanical; fungus? Mechanical	Worldwide Andes
Potyviridae	PVY, PVA, PVV WPMV	740 x 11	Aphids Unknown	Worldwide Peru
Rhabdoviridae	PYDV, EMDV	380 x 75	Leafhopper	USA, Iran
Tobamovirus	TMV, 14R	300 x 17	Mechanical	Andes, USA
Furovirus	PMTV	10-150 x 18-20	Fungus	Europe, Andes
Tobravirus	TRV	190 x 22, 45 X 22	Nematode	Europe, USA
Trichovirus	PVT	640 x 10	Mechanical, seed	Andes
Tymovirus	APLV	28 (diam.)	Beetles	Andes

*Acronyms are those accepted by The International Committee on Taxonomy of Viruses (ICTV). AMV, alfalfa mosaic; PYV, potato yellowing; TSWV, tomato spotted wilt, PVS, potato virus S; PVM, potato virus M; PVP, potato virus P; PRDV, potato rough dwarf; APMoV, Andean potato mottle; PYVV, potato yellow vein; CMV, cucumber mosaic; BCTV, beet curly top; PYMV, potato yellow mosaic; SALCV, *Solanum* apical leaf curling; TSV, tobacco streak; PLRV, potato leafroll; SYV, *Solanum* yellows; TNV, tobacco necrosis; PBRV, potato black ringspot; TRSV, tobacco ringspot; AVB (O), arracacha virus B strain Oca; PVU, potato virus U; PVX, potato virus X; PAMV, potato aucuba mosaic; PapMV, papaya mosaic; PepMV, pepino mosaic; PVY, potato virus Y; PVA, potato virus A; PVV, potato virus V; WPMV, wild potato mosaic; PYDV, potato yellow dwarf; EMDV, eggplant mottle dwarf; TMV, tobacco mosaic; 14R, 14R virus; PMTV, potato mop-top; TRV, tobacco rattle; PVT, potato T, and APLV, Andean potato latent viruses.

Although viruses infecting potato plants are seldom lethal, some viruses cause considerable reduction in crop yield and quality (Beemster and de Bokx 1987). For example, planting tubers of a susceptible cultivar containing severe strains of *Potato virus Y* (Family *Potyviridae*, genus *Potyvirus*, PVY) or PLRV can lead to rapid spread of these viruses throughout the crop, the development of severe symptoms and yield losses of up to 80% (Banttari *et al.* 1993). In contrast, infection with PVX may not result in visible symptoms, but may reduce yield by 10-30% (Banttari *et al.* 1993). Some viruses are relatively benign by themselves, but may in combination with others cause much larger losses in yield. For example, PVS and PVX are generally considered to cause yield losses of up to 10-20% and 15-20% as single infections respectively, but may cause losses of up to 40% when they occur together as co-infections with each other or with some other viruses (Stevenson *et al.* 2001). The effects of viruses on potato growth and yield are influenced by many factors including the virus species, virus strain, resistance of the potato cultivar, growth stage of the plant at the time of infection and environmental conditions (Banttari *et al.* 1993).

PVS and PVX are two of the most common viruses infecting potato worldwide and are widespread in potato growing regions (Jones 1983; Brunt and Loebenstein 2001). Although PVX was not described in the literature until 1931, PVX-like symptoms were described in Tasmania in 1929 (Oldaker 1929). PVS was originally detected in Tasmania in 1968 (P. Cross, DPIW, *personal communication*), respectively, but were considered to have been eradicated from commercial crops. However PVS and PVX were recently detected in commercial seed crops in Tasmania. This is of concern to the local industry as: a) their presence at levels above the Australian National Standard for Certification of Seed Potato prevents the certification and export of seed

potato interstate and overseas; b) there are potential deleterious effects on yield, and c) indicates a failure in the certification scheme.

This review will provide information on potato production in Tasmania and on PVS and PVX with respect to their characteristics, detection, epidemiology, impact on potato production and control.

2.1.4 Potato production in Tasmania

Potato has been an important agricultural industry in Tasmania for over two centuries. Early records suggest planting of potato in Tasmania occurred at Researche Bay by French explorers in 1790's. Potatoes were also planted on Bruny Island in 1792 by Captain Bligh. Captain Bligh recorded the loss of all potato plants from this crop, however, he noted in his diary after leaving Bruny Island that maybe he should have searched under ground. The first reported potato harvest in Tasmania occurred in 1803, planted by Lieutenant John Bowen at Risdon Cove (Taylor 2003).

Currently, the major potato-growing region in Tasmania occurs in a wide band stretching the length of the north coast, ranging from the north-east, Dorset municipality to south of Launceston through to the municipality of Circular Head in the north-west (Figure 2.1).

Figure 2.1. Major potato growing areas of Tasmania (represented by red dots), with the majority of the potato production occurring along a coastal band of the North-West Coast of Tasmania.

Approximately 25% of the total potato crop produced in Australia is grown in Tasmania. The Tasmanian potato industry is estimated to be worth A$89 M at the farm gate in 2004/2005, making it the most valuable vegetable/fruit crop produced in the State. The majority of potato crops are grown for processing by Simplot Australia Pty. Ltd., and McCain Foods (Australia) Pty. Ltd (Anon. 2006a). Approximately 90% of the total 480 000 tonnes estimated to be produced in Tasmania annually are utilised

for French fry production (Kirkwood 2003b). There has been a steady increase in the mean yield/ha recorded annually in Tasmania (Table 2.3) (Anon. 2006b).

Table 2.3. Potato production in Tasmania, years ending 30 June (from Anon. 2006b).

	Production '000 t	Area '000 ha	Mean Yield t/ha
1997(a)	317.4	7.4	42.7
2001	331.0	7.5	44.0
2002	350.1	7.4	47.1
2003	320.3	6.5	49.4
2004	327.6	6.8	48.4
2005	320.8	6.7	47.8

(a) Year ended 31 March

Climatic conditions in Tasmania are ideal for the production of potatoes. Moderate daytime temperatures of around 20°C are favourable for plant growth and the development of tubers. Optimum yields are achieved with high daytime temperatures (25-30°C) in conjunction with low night temperatures (approx. 15°C). Photosynthetic rates increase during the day as a result of the high daytime temperature. In addition, the effect of the low night temperature results in reduced respiration and this enhances the transport of assimilate to tubers (Rabinowitch and Levy 2001).

The main potato cultivars grown in Tasmania include Russet Burbank (late season cultivar), Ranger Russet (mid to late season), Shepody (early to mid season), and to a lesser extent Kennebec (early season). The expected date of harvest, the probability of frosts and weather conditions govern planting time. In general, planting of early-maturing cultivars and mid-season cultivars commences in late May and August

respectively. The planting of late maturing cultivars occurs from mid-September onwards (Kirkwood 2003c), with the majority of the crop in Tasmania planted between early October and early November. Harvest of the potato crop starts in late January and continues until August or September, depending on weather conditions.

Figure 2.2. Potato field in Tasmania cv. Russet Burbank (photo taken January 2003 at Scottsdale).

Although several potato cultivars are grown in Tasmania, Russet Burbank is the main processing cultivar. Russet Burbank possesses desirable potato processing characteristics including tuber shape, specific gravity and fry colour. Tuber shape is preferably long and block shaped to minimise waste at the factory. Specific gravity (SG) of a tuber influences French fry texture and is a measure of density. Optimal fry

colour for processing is very light golden brown and is related to the quantity of sugar in the tuber (Kirkwood 2003b).

2.2 *Potato virus S* (PVS)

2.2.1 Characteristics, distribution and symptoms

Potato virus S (PVS) is a member of the genus *Carlavirus* (Wetter 1971). PVS particles are slightly flexuous, rod-shaped, range in size between 610-700 nm long by 12-13 nm wide and consist of 95% protein and 5% linear single-stranded ribonucleic acid (RNA) (Brunt and Loebenstein 2001; Stevenson *et al.* 2001). The viral genome consists of one single-stranded positive sense RNA molecule, approximately 7.5 kb in size, which contains a 3'-terminal polyadenylated region (Monis *et al.* 1987, Mackenzie *et al.* 1989) (Figure 2.3).

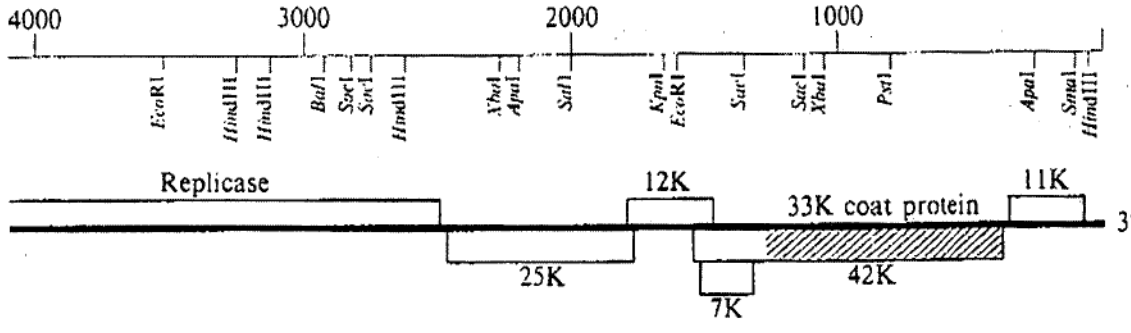


Figure 2.3. The genome organisation and restriction endonuclease map of 3'-terminal region of PVS RNA (Mackenzie *et al.* 1989).

The RNA is encapsidated in an approximately 34-kDA coat protein (Mackenzie *et al.* 1989, Foster and Mills 1992, Monis *et al.* 1987). Translation of PVS RNA *in vitro* in rabbit reticulocyte lysate, yields primarily four products with M*r* values of 124K, 112K, 98K and 36K (Monis *et al.* 1987). PVS forms minor amounts of two subgenomic RNAs (2.5 and 1.5 kb) which are contained within the virus particles, the smaller of which codes for the coat protein and the 11-kDA protein (Foster and Mills 1992). Turner *et al.* (1999) proposed that this subgenomic RNA was able to greatly enhance translation.

Carlaviruses such as PVS typically possess a limited host range and tend to induce mild or no symptoms in host plants. Viruses that do not induce symptoms in a host plant are termed "latent" viruses (Foster 1991). Although the first report of PVS in potato crops occurred over 50 years ago in Holland (de Bruyn Ouboter 1952), it is likely to have occurred previously in other potato growing countries (Foster 1991; Brunt and Loebenstein 2001). PVS is found widespread in many countries and is considered one of the most common potato viruses in potato production (Jones 1983; Brunt and Loebenstein 2001). In both Canada and the United States, PVS is considered the fourth most important potato virus, preceded by PVX (ranked 3rd), PVY (ranked 2nd) and PLRV (ranked 1st) (de Boer *et al.* 1996).

2.2.2 Strains

Symptoms

Disease symptoms in potato crops caused by PVS vary and depend upon the strain of PVS present, and also the cultivar. Infected plants often appear healthy. However,

where symptoms occur, they include slight deepening of veins, rugosity of leaves, stunting of plants, more open growth habit, and mottling, bronzing and necrotic spots on upper leaves of some cultivars (Stevenson *et al.* 2001). Infected potato plants may also exhibit premature senescence of older leaves (Dr. R.A.C. Jones, Department of Agriculture and Food Western Australia (DAFWA), *personal communication*). Symptom expression of PVS in the field may be enhanced during cloudy weather compared to sunny weather conditions (Beemster and de Bokx 1987). The poor symptom expression caused by PVS infection in potato means that infected plants can be overlooked during visual inspection of crops.

Distribution

Two major strains of PVS are recognised, the ordinary strain (PVS^O) and the Andean strain (PVS^A), along with several minor strains (Brunt 2001b). Until the 1980s, it was considered potato cultivars infected with PVS worldwide contained only minor PVS variants (Wetter 1971). These isolates were characterised as causing only local lesions following mechanical inoculation to the indicator host *Chenopodium quinoa* (Figure 4.4). For example, Slack (1981) reported that at 8-12 days following inoculation of *C. quinoa* with PVS strains found in Europe and North America, local infection with characteristic chlorotic lesions appeared (Slack 1981). However, Slack (1981) found an aphid transmitted strain of PVS in the USA which caused both a typical localised symptom in *C. quinoa*, along with systemic symptoms. In the early to mid 1980s novel strains of PVS were also reported in the Netherlands (Rose 1983), the U.S.A. (Slack 1981; Jones 1983), the U.K. (Slack 1981) and Germany (Slack 1983; Dolby and Jones 1987). These strains of PVS and strains from South America were found to cause local and systemic lesions when inoculated into *C. quinoa* (Hinostroza-Orihuela

1973; Santillan 1979; Brunt and Loebenstein 2001). The term 'Andean strain' (PVS^A) has become generally used to describe those strains of PVS which are systemic in *C. quinoa* and 'ordinary strain' (PVS^O) to describe those which cause only local lesions (Jones 1981, Slack 1983, Dolby and Jones 1987). The introduction and subsequent establishment of PVS^A outside the Andes may have resulted from escape from imported potato germplasm (Jones 1983). Both PVS^A and PVS^O are readily transmitted mechanically by plant to plant contact, however PVS^A has been shown to be more easily transmitted by aphids than PVS^O (Hinostroza-Orihuela 1973; Slack 1981; Brunt and Loebenstein 2001). The specific identification of PVS^A is now possible due to the production of monoclonal antibodies for this strain (Cerovska and Filigarova 1995; Brunt and Loebenstein 2001).

a) **b)**

Figure 2.4. Symptom expression of the Andean strain of *Potato virus S* (PVS^A) on inoculated leaf of *Chenopodium quinoa* at three weeks post inoculation (a) and systemic faint mottling symptoms (b) (specimens kindly provided by John Fletcher, New Zealand Crop and Food Research, photo taken 28/07/2005, Lincoln, N.Z.).

PVS^A has also been reported in New Zealand (Fletcher 1996) where it was transmitted at low efficiency by aphids, suggesting that mechanical transmission was more important. PVS^A has also been shown to infect Pepino (*Solanum muricatum*) and

infection of this host has been reported in the Netherlands and New Zealand (Jones 1983; Brunt and Loebenstein 2001). In addition, PVS^A has occasionally been reported in several weed host species including *Solanum chacoense*, *S. brevidens*, *S. spegazzini* and *S. dulcamara* (Valkonen *et al.* 1992 cited in Brunt and Loebenstein 2001).

Molecular relationship between strains

Foster *et al.* (1990) used complementary deoxyribonucleic acid (cDNA) solution hybridisation to compare sequence homologies between RNAs of 5 isolates of PVS^O and 4 isolates of PVS^A. They found a high degree of sequence homology (90-100%) between the majority of PVS^O and PVS^A isolates. MacKenzie *et al.* (1989) sequenced the 3' terminal region of PVS^A. Weidemann and Koenig (1990) compared isolates of PVS^A from the Andean region and from Germany that were able to infect *C. quinoa* systemically. Isolates were unable to be differentiated by the serological technique, enzyme-linked immunosorbent assay (ELISA) using polyclonal antisera, indicating that they were serologically closely related. However, differences were noted between the isolates using quantitative cDNA hybridisation. Weidemann and Koenig (1990) also demonstrated biological differences between strains, with PVS^A from the Andes successfully aphid-transmitted by *Myzus persicae* (Sulz.) to 36/426 (8.5%) *S. demissum* plants, while the strain from Germany was transmitted to only 5/210 (2.4%) plants in aphid transmission studies. Dolby and Jones (1987) also showed differences between isolates in symptoms produced in test plants and potatoes. Weidemann and Koenig (1990) concluded that the use of the term 'Andean strain' was somewhat misleading as it consisted of a number of heterogeneous strains.

Matoušek *et al.* (2000) used more sensitive techniques to investigate the sequence variability of Central European isolates of PVS^O, which were unable to develop systemic infection in *C. quinoa*. A phylogenetic tree based on the coat protein amino acid sequences demonstrated greater than 96.7% homology between isolates. The 3' terminal portion of one of these isolates was sequenced and was shown to have 81.4% homology with PVS^A, along with some shifts in open reading frame patterns. The most significant differences were at the N-terminal regions of the 7-kDA protein, coat protein and 11-kDA protein encoded by the 3' terminal region of the genome, suggesting these could be responsible for differences in virus movement and symptom development between PVS^O and PVS^A. More recently Matoušek *et al.* (2005) reported the complete nucleotide sequence of PVS strains which were both non-systemic and systemic in *C. quinoa* and presumably PVS^O and PVS^A, respectively.

Diagnostic indicator species

Indicator plants are plant species that produce characteristic symptoms when inoculated with particular viruses. They are useful tools in determining which virus or which strain of a virus is present within a host plant (Randles and Ogle 1997). Although the natural host range of PVS is very limited, experimental transmission can be attained by mechanical inoculation to many species including in excess of 56 additional solanaceous species and 33 species from 12 different families (Brunt and Loebenstein 2001). Symptoms induced by PVS infection on particular indicator species are outlined in Table 2.4.

Table 2.4. Indicator species of *Potato virus S* (PVS) (from Brunt and Loebenstein 2001; Fletcher 1996).

Experimental Host	Symtoms
Chenopodium album,	Chlorotic local lesions, often with green halo on older leaves.
C. amaranticolor	Chlorotic local lesions and PVS^A inoculation result in systemic necrotic lesions and yellow vein banding
C. quinoa	PVS^O induces chlorotic local lesions, often with green halo on older leaves. PVS^A inoculations result in systemic infections and characteristically induce chlorotic spotting.
Cyamopsis tetragonoloba	Small brown necrotic lesions in inoculated cotyledons, but no subsequent systemic infection.
Lycopersicon esculentum	Symptomless systemic infection by PVS^A but immune to PVS^O
Nicotiana clevelandii	Conspicuous chlorosis of systemically infected leaves. Use host for maintenance and propagation of virus cultures.
Nicotiana debneyi	Symptomless local infection but vein-clearing, mottling and necrosis of systemically infected leaves. Useful in the separation of PVS and PVM (immune to PVM).
Solanum rostratum	Numerous small necrotic lesions in inoculated and systemically infected leaves.
Nicotiana benthamiana	Symptomless infection

A new carlavirus, *Potato latent virus* (Family *Flexiviridae*, genus *Carlavirus*, PotLV) has recently been described in potato. This virus was previously referred to as Red la Soda virus, and has similar characteristics to PVS^A, although systemic infection occurs in different diagnostic species (Goth *et al.* 1999). For example one diagnostic host for PotLV is *Nicotiana tabacum* which is not a host for PVS (Brattey *et al.* 2002). PVS may be found in association with the related carlavirus, *Potato virus M* (Family *Flexiviridae*, genus *Carlavirus*, PVM) in some cultivars of potato. The two viruses can be separated diagnostically by inoculation of the indicator species, *Nicotiana debneyi*, in which PVS infects the plant systemically and PVM causes a local infection (Brunt *et al.* 1996). Separation of PVS from PVM has been reported by some authors through the apparent inability of the former to infect tomato (*Lycopersicon*

esculentum) (Bagnall *et al.* 1956, Wetter 1972). However, other authors have shown some strains of PVS are capable of infecting *L. esculentum* (Slack 1983).

2.2.3 Transmission

As discussed previously, transmission of PVS occurs commonly by mechanical means. However, some strains of PVS are also transmitted by aphids in a non-persistent manner.

Mechanical transmission

PVS is considered adequately infectious for mechanical transmission to occur between infected and healthy field-grown potatoes (Franc and Banttari 1984; Brunt and Loebenstein 2001). Mechanically transmitted viruses are unable to penetrate through an intact plant surface so the virus must enter through a wound on the plant surface in order to facilitate entry into cells (Franc and Banttari 2001). Approximately 10^4 to 10^5 PVS particles are reportedly required to be applied to a leaf surface for successful mechanical inoculation (Matthews 1970; Franc and Banttari 2001). PVS is not transmitted via true seed (Goth and Webb 1975).

Transmission by seed cutting

In Tasmania prior to commercial potato planting, large tubers are cut into smaller pieces by either a hand cutting or machine cutting process (Figure 2.5). Seed cutting has been shown to be a source of PVS transfer (Franc and Banttari 1984). The importance of stringent sanitation procedures and indexing to produce seed potatoes free from PVS was highlighted in a study conducted by Franc and Banttari (1984). In

Russet Burbank, transmission of PVS from infected to uninfected tubers via the hand seed cutting process increased significantly if the cutting knife passed through a sprout of a tuber (45.2%) compared to knife contact with non-sprout tuber tissue (24.5%). Franc and Banttari (1984) demonstrated that transmission efficiency of PVS by seed cutting differed between cultivars. Cutting infected tubers followed by healthy tubers led to 76.7% and 62.6% infected plants for Russet Burbank and Kennebec respectively, significantly higher (P=0.01) than for cultivar Norland (25% infection).

Figure 2.5. Centralised mechanical seed cutting machine located at Spreyton, Tasmania (photo taken October 2003).

Opinions regarding seed damage during seed-cutting operations caused by mechanised or hand-cutting blades vary in the potato industry. On the one hand it is suggested that seed cut by hand may possess a ragged cut surface compared to a tuber cut by machine, thus facilitating virus transfer. Alternatively, mechanised blades may

result in greater tissue damage due to the crushing force of the blades (Sturz *et al.* 2000). A study to evaluate the effect of PVY ordinary strain (PVY^O) transmission of both hand and machine cut seed found no evidence to support mechanical transmission through either form of cutting method (Sturz *et al.* 2000). The difference between hand cutting and machine cutting on transmission of PVS between tubers has not been studied.

A recent study has shown PVS replication within the tuber is induced when potato tubers are mechanically wounded (Morelli and Vayda 1996). Morelli and Vayda (1996) suggested that stimulation of macromolecular metabolism is induced by wounding of the tuber, which stimulates viral replication. Poor handling of tubers prior to cutting might therefore stimulate PVS replication and increase virus transmission between tubers during the cutting procedure.

Retention of activity in the absence of host plants

Franc and Banttari (1984) found PVS to remain infectious for 120 hr and 204 hr, where sap from PVS infected plants was maintained in beakers with buffer absent and buffer present, respectively. PVS particles can remain viable in sap for 72-96 hours (Brunt *et al.* 1996). PVS particles were not viable on unpainted wood after a period of 180 hr at 4°C and 100% relative humidity (Franc and Banttari 1984), however PVS particles have been shown to remain viable for over 120 hours on some surfaces (Table 2.5) (Banttari *et al.* 1993).

Table 2.5. Retention of infectivity (in hours) by *Potato virus S* (PVS) on various materials (from Banttari *et al.* 1993).

Material	PVS
Iron or aluminum foil	7
Unpainted wood	0
Painted wood	-
Burlap	120
Cotton	-
Soil	25
Rubber	25
Human skin	-
Expressed sap from potato foliage	120

Aphid transmission

Some strains of PVS are transmitted by some aphid species in a non-persistent manner (Slack 1983). Non-persistent transmission is characterised by rapid acquisition of virus by the feeding aphid (usually less than 30 seconds of feeding), an ability to transmit to a healthy plant after a similarly short period of feeding, and the retention of the ability to transmit for periods of only a few minutes to several hours. Subsequent feeding of the aphid on healthy plants will remove virus particles from the stylets and/or foregut and render the aphid non-viruliferous until it feeds on an infected plant again.

Of the PVS isolates that are aphid-transmissible, aphid species acting as vectors include *Aphis nasturtii* (buckthorn aphid), *A. fabae*, *Rhopalosiphum padi* L. (bird

cherry/oat aphid) and *Myzus persicae* Silz. (green peach aphid) (Brunt and Loebenstein 2001). Of these, *R. padi* and *M. persicae* are present in Tasmania (L. Hill, DPIW, *personal communication*). *A. nasturtii* and *M. persicae* are known to colonise potato (Singh *et al.* 1989). However the range of species tested as vectors of PVS is limited and there may be other, as yet unknown, vector species. For example, *Capitophorus eleaeagni* was recently identified as a potentially important aphid vector of PVY in Idaho (Halbert *et al.* 2003). A summary of aphid transmission of PVS studies are listed in Table 2.6.

A study conducted by Singh *et al.* (1989) monitored the spatial pattern of PVS infection in a field of healthy plants over two consecutive seasons (1986 and 1987). PVS was detected in the later stages of the crop development, however, the presence of PVS could not be attributed to within field spread of infection, due to the absence of PVS source plants in the test field in addition to individual plants being well separated. PVS detection in this study coincided with a flight period of both *A. nasturtii* and *M. persicae* thereby implicating aphid spread. However, the evidence for aphid transmission was considered circumstantial (Singh *et al.* 1989). Wardrop *et al.* (1989) showed that under controlled conditions both aphid species transmitted the same PVS isolate, thus providing further evidence for aphid transmission in the field (Singh *et al.* 1989; Wardrop *et al.* 1989).

Table 2.6. Summary of studies into aphid transmission of *Potato virus S* (PVS).

Author	Virus Strain	Aphid species	Source of virus	Assay host	No. with virus/no. plants inoculated
Slack (1983)	PVS^A	*Myzus persicae*	Potato	Potato	7/69 (10.1%)
				Potato	0/30
			C. quinoa	*C. quinoa*	3/23 (13.0%)
			Purified	Potato	7/26 (26.9%)
				C. quinoa	0/25
	PVS^O	*M. persicae*	Potato	Potato	0/103
				Potato	0/30
			Purified	Potato	0/29
Fletcher (1996)	PVS^A	*M. persicae*	Potato	*C. quinoa*	0/10
				Potato	0/10
	PVS^A	*Aulacorthum solani*		Potato	1/10 (10%)
	PVS^O			Potato	0/10
	PVS^A	*M. persicae*		Potato	0/10
	PVS^O			Potato	0/10
	PVS^A	*M. persicae*	Purified	Potato	1/9[1] and 2/9[2] (11.1 and 22%, respectively)
				C. quinoa	2/5[1] and 2/5[2] (40% each)
Weidemann and Koenig (1990)	PVS^A				36/426 (8.5%)
	PVS^O				5/210 (2.4%)

Table 2.6. Summary of studies into aphid transmission of *Potato virus S* (PVS) cont.

Author	Virus Strain	Aphid species	Source of virus	Assay host	No. with virus/no. plants inoculated
Weidemann (1986)					2.9%
Santillan (1979)	PVS[A]	*M. persicae*		*C. quinoa*	Up to 40-50%
MacKinnon (1974)		*M. persicae*		*S. demissum*	3.4%
Wardrop *et al.* (1989)	PVS	*M. persicae* (alatae)	Potato	Potato	[3]RP=1/172 (5.9%), RB=0/17, Shep=1/16 (6.3%), Seb=1/15 (6.6%)
		M. persicae (apterae)			[3]RP=2/17 (11.8%), RB=0/17, Shep=2/16 (12.5%), Seb=2/14 (14.3%)
		Aphis nasturtii (alatae)			[3]RP=1/17 (5.9%), RB=0/17, Shep=0/16, Seb=0/15
		A. nasturtii (apterae)			[3]RP=1/17 (5.9%), RB=1/17 (5.9%), Shep=1/16 (6.3%), Seb=0/14

[1] aphid feeding for 1 hour or overnight[2] on assay host

[3] RP= cv. Red Pontiac, RB= cv. Russet Burbank, Shep= cv. Shepody, Seb= cv. Sebago.

Two of the critical factors with regard to aphid vector/virus dynamics are the relative contribution of colonising versus non-colonising aphids and the role of virus sources within the crop (e.g. from infected seed) versus virus sources outside the crop (e.g. weed hosts). While little is known with regard to PVS, several studies have been conducted on PVY, which is transmitted in a similar non-persistent fashion by some aphid species. Studies in the USA by Harrington *et al.* (1986), Boiteau *et al.* (1988) and DiFonzo *et al.* (1996a) have suggested that winged non-colonising aphids coming from outside of the crop are responsible for the majority of PVY infection in potato fields. However, van Hoof (1979) found a significant correlation between the abundance of *M. persicae* in the crop and local PVY spread. Similarly, Harrewijn *et al.* (1981) suggested that short hovering flights of *M. persicae* summer migrants could be responsible for significant spread. Halbert *et al.* (2003) identified the non-colonising aphid *Capitophorus elaeagni* as a vector of PVY and showed it to make up 18.5-31.7% of pan trap catches in potato fields in Idaho, USA, suggesting a strong propensity to land in potato fields. Halbert *et al.* (2003) suggested that *C. elaeagni* was an important vector of PVY in Idaho. Similarly, several cereal aphids (e.g. *Rhopalosiphum padi*), which were known vectors of PVY, were captured in potato fields in Idaho. Although the transmission efficiency of cereal aphids of PVY is not as high as *M. persicae*, they were much more abundant in seed potato fields in Idaho, suggesting that they were more important vectors of PVY (Halbert *et al.* 2003). Ragsdale *et al.* (2001) also indicated the *R. padi* was the most important vector of PVY in Midwestern USA. Non-colonising aphids may be particularly good virus vectors in that they may move between potato plants as they sample for a preferred host and spread virus as they do so. Radcliffe *et al.* (1993) noted that where the source of virus originated from plants growing from a few infected seed pieces rather

than from outside the field, the prevention of build-up of the aphid population was important in control, regardless of whether winged or wingless aphids were involved. In areas of seed production in North America, migration of *M. persicae* typically occurs toward the end of the season (de Boer *et al.* 1996). In some parts of the United States aphid migration is detected using aphid pan traps in fields, and used to inform farmers when to begin aphicide applications (Satapathy 1998; Sigvald 1998, Radcliffe *et al.* 1993).

2.2.4 Movement of PVS within the potato plant

Franc and Banttari (2001) summarised much of the literature on movement of PVS within the potato plant. Franc and Banttari (1996) reported that PVS moved out of mechanically inoculated leaves within 24 hours, with virus detectable by ELISA in foliage above and below the inoculated leaf within 13 and 20 days respectively. PVS was translocated from inoculated leaves to daughter tubers within 13 days for Russet Burbank and Norland, and 20 days for Kennebec and within 14 days for European cultivars (de Bokx 1968, de Bokx and Waterreus 1967). de Bokx (1968) reported mature plant resistance to PVS in European cultivars at four weeks after planting, which became more pronounced at six weeks. The degree of resistance varied with cultivar and with PVS isolate. However, Franc and Banttari (1996) were unable to detect mature plant resistance to a Minnesota isolate of PVS and postulated that this at least partially explained the rapid reinfection of healthy potatoes in Minnesota. Spread of European isolates of PVS to tubers would be limited to the early part of the season and become more limited with the onset of mature plant resistance, thus reducing reinfection in seed-lots in European production areas even when inoculum

was present. By contrast, the lack of mature plant resistance to Minnesota isolates of PVS would allow spread from infected plants to tubers throughout the season, especially late in the growing season when contact between foliage and stems became more pronounced and the likelihood of mechanical transmission from plant to plant was increased and perhaps coinciding with late season flights of aphids (de Boer *et al.* 1996).

2.2.5 Resistance to PVS

Some potato cultivars, such as the Canadian cultivar Jemseg (Bagnall 1988a) and the cultivar Saco (Khurana and Garg 1998) are field immune to PVS and the resistance is inherited as a recessive. Hypersensitive resistance is found in *S. tuberosum* ssp. andigena and in *S. megistacrolobum* and is regulated by the *Ns* gene (Bagnall 1988a). Following challenge with PVS, plants with the *Ns* gene remain symptomless and no detectable PVS develops in the plant. Following graft inoculation of infected plants, the resistant plants react with a fading of the foliage of shoots developed from the axillary meristem as a result of the hypersensitive response (Marczewski *et al.* 2002). Marczewski *et al.* (2002) mapped the *Ns* resistance gene to potato chromosome VIII. Resistance to PVS has also been found in accessions of *S. brevidens* (Anon. 1991). Genetic engineering has been used to develop transgenic *N. debneyi* plants expressing the coat protein gene of PVS (PVS-CP). These plants were highly resistant to infection by PVS, with an absence of symptoms and a lack of virus accumulation in both the inoculated leaves and other parts of the plants (Mackenzie and Tremaine 1990).

2.3 *Potato virus X* (PVX)

2.3.1. Characteristics, distribution and symptoms

PVX was first described in the UK by Smith (1931). PVX is distributed worldwide in potato growing areas (Bercks 1970; Beemster and de Bokx 1987) and occurs in all Andean countries (Jones 1983; Brunt and Loebenstein 2001). PVX particles are flexuous and rod-shaped with a length of 515 nm and width of 13 nm (Stevenson *et al.* 2001). Several synonyms of PVX include potato mosaic virus (Brunt *et al.* 1996), potato mild latent virus, *Solanum* virus 1, potato latent virus, healthy potato virus and potato virus 16 (Beemster and de Bokx 1987). Other diseases caused by PVX infections in potato include potato inter-veinal mosaic, potato simple mosaic and potato top necrosis. Tuber size and quantity may be compromised by PVX infection in potatoes, and result in slightly fewer and smaller tubers compared to healthy plants (Beemster and de Bokx 1987).

Symptoms in potato crops resulting from PVX infection are variable and influenced by many factors including virus strain, variety and the presence of mixed viral infections. Environmental conditions also impact on the symptomatology of PVX in potato (Loebenstein 2001). Typically many PVX isolates evoke mild symptoms and for this reason PVX was for many years referred to as the 'healthy plant' virus and considered practically harmless to potato plants. Generally, symptomless (latent) or mild leaf mosaic symptoms result from infection by the majority of PVX isolates in potato, particularly at elevated temperatures. Although plants may possess high virus titre, generally, less than 10% yield loss have been reported due to PVX (Brunt and

Loebenstein 2001). At higher temperatures, some isolates induce little or no symptoms. An interveinal mosaic response can occur with infections by some PVX strains, with a visible mosaic pattern between leaf veins. This is generally more visible at low temperatures (16-22 °C). At temperatures over 22 °C, symptoms caused by infections with the same strain are less pronounced (Beemster and de Bokx 1987; Draper *et al.* 2002). Infected plants that display mild symptoms of PVX in the upper leaves show characteristic symptoms in the older leaves. In healthy plants, older leaves shaded by top foliage generally become uniformly yellow. However, the older leaves of plants possessing mild symptoms of PVX (in upper leaves) tend to possess pronounced greenish veins and the remainder of the leaf turns yellow (Beemster and de Bokx 1987). Symptoms of PVX are commonly observed in low light intensity (cloudy conditions) (Draper *et al.* 2002).

Virulent strains of PVX may cause more severe symptoms such as severe mosaic, necrotic streak, rugosity or crinkling of leaves and may result in considerable yield losses (Beemster and de Bokx 1987; Brunt and Loebenstein 2001), dwarfing, reduced leaflet size or extensive necrosis of tops and tubers (Stevenson *et al.* 2001). Co-infections of mild strains of PVX with PVA or PVY can also lead to a severe mosaic with crinkling, rugosity, or necrosis of leaves. Plants infected with PVX that remain symptomless are referred to as carriers. Identification of potential carrier varieties in which PVX is latent is important to the seed potato industry, as these carriers provide an infection source for cultivars that display severe reactions to infection. The ability to prepare a high-titre antiserum against PVX has allowed the detection of PVX in tubers (sprouted or dormant) and foliage by means of the serological technique, ELISA or methods of precipitation or agglutination (Beemster and de Bokx 1987).

2.3.2 Natural host range

PVX has a limited natural host range constrained mainly to solanaceous species (Bercks 1970) such as *Solanum nigrum*, *S. tuberosum*, *Nicotiana* spp., *Petunia hybrida*, *Datura stramonium*, *Cyphomandra betacea* and *Lycopersicon esculentum* (Brunt and Loebenstein 2001; Ali and Hassan 2002). Other susceptible families include Chenopodiaceae and Amaranthaceae. As discussed above, symptoms in potato can vary. Some strains of PVX are symptomless in *S. tuberosum*, while severe necrotic streaks are induced by other strains (Bercks 1970). Symptoms of PVX-infected plants of *Brassica campestris* spp. *rapa* include stunted plants, leaf distortion and mild mosaic mottling (Brunt *et al.* 1996).

2.3.3 Strains and host resistance

Different researchers have divided PVX strains into different groupings according to various properties including, serological reactions (Matthews 1949) and thermal inactivation point. A widely used method of differentiation is into four groups based on their ability to overcome resistance conferred by two dominant resistance genes Nx and Nb in *S. tuberosum* (Cockerham 1955).

Grouping of PVX strains is based on a hypersensitive reaction (HR). This is induced by group 1 strains in potatoes possessing either genes Nb or Nx, by group 2 strains in potatoes with the *Nb* gene, by group 3 strains in potatoes with the Nx gene (Table 2.7). Resistance provided by *Nb* and *Nx* gene mediation can be overcome by the strains in group 4 (Cockerham 1955). A recent review of host major gene resistance

to potato viruses including PVX was provided by Solomon-Blackburn and Barker (2001a).

Table 2.7. Genes for the hypersensitivity reaction in potato and the reaction of different strain groups of *Potato virus X* (PVX) (from Cockerham 1955).

Cultivar	Genotype		Strain Group			
			1	2	3	4
Arran Banner	nx	nb	s	s	s	s
Epicure	Nx	nb	R	s	s	s
Arran Victory	nx	Nb	R	R	s	s
Craigs Defiance	Nx	Nb	R	R	R	s

R- hypersensitivity; s- susceptible

Group 1 PVX strain is reportedly widespread in Australia, while in the UK, group 3 is more widespread (Wilson and Jones 1995; Brunt and Loebenstein 2001). Other strains have also been described. Extreme resistance to PVX was first observed in the clone USDA 41956 (Schultz and Raleigh 1933) and is controlled by the gene Rx(adg) which is found in accessions of *S. tuberosum* ssp. *andigena* and in material often used in the Dutch breeding program for nematode resistance (Khurana and Garg 1998). Another gene causing extreme resistance is found in *S. acaule* (Ross 1954). Some 23 European potato cultivars carry extreme resistance to PVX, but the resistance is temperature dependent (Khurana and Garg 1998). A Bolivian PVX strain, (X^{HB}) is able to overcome resistance conferred by the single gene Rx. (Moreira *et al.* 1980; Jones 1983; Jones 1985). The discovery of PVX^{HB} in 7% of *S. tuberosum* spp. *andigena* cloned in Bolivia is problematic for the potato industry (Moreira *et al.* 1980;

Jones 1985; Brunt and Loebenstein 2001). The principle determinant for the ability of PVX strains to overcome resistance conferred by the genes Nx and Rx1 has been attributed to the viral coat protein (Kavanagh *et al.* 1992; Brunt and Loebenstein 2001). Avirulent strains of PVX to potato plants that have Rx1 resistance possess a threonine residue on the coat protein at position 121, while lysine residue is present at the same position of the coat protein of PVX^{HB}, suggesting that this is associated with the ability of this strain to overcome Rx1 resistance in potatoes (Querci *et al.* 1995 cited in Brunt and Loebenstein 2001).

Potato cultivars possessing the resistance gene, Nx have been shown to produce a hypersensitive reaction when infected with strains of PVX DX. The Nx-mediated resistance may be overcome by a natural mutant of the PVX DX strain, PVX DX4 (Jones 1982; Brunt and Loebenstein 2001). Sequence comparison between PVX DX strain and the mutant strain PVX DX4 showed the coat protein of the latter possessed a glutamine 78 substitution for proline, enabling the avoidance of the hypersensitive reaction in cultivars containing the Nx gene (Goulden *et al.* 1993; Brunt and Loebenstein 2001). Conversely, a proline 78 substitution was not detected in PVX MS from Argentina. PVX MS was suggested to be similar to PVX^{HB} (Feigelstock *et al.* 1995 cited in Brunt and Loebenstein 2001).

Different PVX strains have been reported to provide complete, partial or the absence of cross-protection of plants to subsequent strain infections (Bercks 1970). Cross-protection refers the protection provided by one virus strain against the invasion and infection of another related strain (Fletcher 1978).

Genetic engineering has been used to develop potato cultivars resistant to PVX. This has been based on a variety of mechanisms including coat-protein gene mediated resistance. This utilises a long observed phenomenon that infection of a plant by one virus or virus strain may prevent infection by another virus or virus strain. The mechanism of this resistance is still not well understood (Khurana and Garg 1998). The commercial potato cultivars Bintje and Escort (Hoekema *et al.* 1989) and Russet Burbank (Lawson *et al.* 1990) were transformed to express the coat protein (CP) gene of PVX. Transgenic plants accumulated PVX-CP at 0.05-0.20% of total plant protein. Inoculation of transformed cultivars Bintje or Escort with PVX caused a 20 to 50 fold decrease in PVX accumulation than untransformed plants and slower symptom development (Hoekema *et al.* 1989). Transformed Russet Burbank plants did not accumulate any PVX at any of three different inoculum concentrations (Lawson *et al.* 1990). In field trials, two of four transformed Russet Burbank lines showed significant protection from infection by PVX (Kaniewski *et al* 1990). Transgenic tobacco lines expressing the replicase gene from PVX have been developed and shown to be resistant when inoculated with PVX (Braun and Hemenway 1992). The Rx gene from the potato cultivar Cara was cloned (Kanyuka *et al.* 1999) and transferred to *Nicotiana benthamiana* to induce resistance against PVX (Bendahmane *et al.* 1999). Similarly, inserting PVX genes associated with virus movement from cell to cell into the potato cultivar Pito led to resistance to PVX and other viruses including PVS, but not PVY (Seppänen *et al.* 1997). Several other strategies for genetically engineering resistance to PVX, PVS and other viruses have proven successful in an experimental capacity (Berger and German 2001). While genetic engineering has considerable potential to reduce the impact of virus infections on potato crops, much more work on assessing risks (e.g. Anon. 2002a) will be required before gaining the necessary consumer and

market approval for widespread application. Genetically engineered potato resistant to PLRV and PVY has been developed in Australia by CSIRO, however are unlikely to be released in the short term. A comprehensive review of traditional and molecular approaches to breeding virus resistant potato was given by Solomon-Blackburn and Barker (2001b).

2.3.4 Transmission

Seed cutting

The transmission of PVX occurs mainly by contact between infected and healthy plants, and is facilitated by mechanical inoculation of sap (Bercks 1970). Sap from infected plants may be carried by workers, clothing, animal fur or farm equipment transmission to healthy plant material through contact (Beemster and de Bokx 1987). In addition, infection may occur in non-infected seed potato tubers during storage as a result of contact between sprouts of infected tubers and non-infected tubers (Brunt and Loebenstein 2001).

PVX has also been shown to be transmitted readily during seed-cutting. Transmission studies by Larson (1950) found a greater transmission frequency of PVX (a ringspot strain) to be transmitted with by the cutting knife when virus infected source tubers were cut through the eyes (52%) compared with cuts through source tubers that avoided eyes (24%).

Equipment and animals

While there have been few quantitative studies, equipment used for cultivation or spraying of crops has been implicated in the spread of PVX. Manzer and Merriam (1961) observed 54-93% infection when PVX contaminated cultivating and hilling equipment were used. Todd (1958) demonstrated that PVX could be spread in the field by walking through PVX infected potato plots and allowing contact between foliage and clothing/boots. Contaminated clothing remained infective for 3 weeks. Rabbits and dogs allowed to run amongst PVX infected potato carried infectious virus for at least 10 days on the back, muzzle and paws of rabbits and for 24 hours on the back, legs and stomach of dogs (Todd 1958). When infected and healthy foliage was handled, the virus was readily acquired and transmitted.

Transmission via plant roots

Roberts (1950) showed root infection of tomato occurred when sap infected with PVX was poured over the soil of potted plants and foliage was prevented from contacting soil. Potato was also infected when roots were directly inoculated with PVX. Roberts (1950) postulated that mechanical injury to roots could allow virus transmission from plant to plant in the field, although it is considered a potentially unimportant means of transmission (Brunt and Loebenstein 2001). Bercks (1970) suggested that PVX may be transmitted between roots of potato by soil borne vectors. Nienhaus and Stille (1965) implicated the fungus *Synchytrium endobioticum* as a potential vector of PVX and Salazar (1966) reported frequent infection of *N. debneyi* and *N. benthamiana* by PVX when planted in soil from the Andes infested by *S. endobioticum*. However, Lange (1978) was unable to transmit PVX from infected potato to tomato plants using zoospores of *S. endobioticum*.

Aerosols

Mechanically transmitted viruses such as PVX may also be transmitted in aerosols (Banttari and Venette 1980). PVX was shown to be transmitted to *Gomphrena globosa* plants that had been subjected to abrasion when aerosols containing PVX were directed through a wind tunnel at moderate air-flow velocities. Infectious aerosols of PVX were also obtained when injured infected tomato and potato plants were subjected to air-blast and water-blast in an enclosed chamber. Naturally formed aerosols of *Tobacco mosaic virus* (Family not assigned, genus *Tobamovirus*, TMV) were detected in a field plot of infected *Nicotiana tabacum*. However, PVX aerosols were not detected during twenty, 24 hour sampling periods in a 40 ha field of highly infected potato (Russet Burbank). TMV occurs at high concentrations in epidermal cells of *N. tabacum*, and the trichomes on *N. tabacum* can be easily broken which may explain the ease with which it was detected in comparison to PVX (Banttari and Venette 1980). However, it was suggested that PVX may be naturally spread in aerosols during more severe weather events including hail, driving rain and wind.

Insects and other means of transmission

Transmission of PVX does not occur through pollen or true seed of potato. PVX is not aphid transmitted, and no specific insect vectors are known (Brunt and Loebenstein 2001). However, several chewing insects, such as planthoppers and grasshoppers, are reported to facilitate transmission between PVX-infected and healthy plants (Brunt and Loebenstein 2001), probably through mechanical transmission. PVX transmission can be facilitated by the grasshoppers, *Tettigonia viridissima* (Schmutterer 1961 cited in Brunt and Loebenstein 2001) and *Melanoplus differentialis* (Walters 1952 cited in Brunt and Loebenstein 2001). However, it is unknown what contribution insects

make to PVX spread in the field. PVX has also been transmitted experimentally by dodder (*Cuscuta campestris*) (Ladeburg *et al.* 1950 cited in Brunt and Loebenstein 2001).

Movement of PVX in the potato plant

The length of time required for transmission of PVX from mechanically inoculated leaflets of several European cultivars of potato into tubers was studied by Beemster (1958). PVX was found in tubers between 2-4 days after inoculation. Generally, the percentage of tubers infected with PVX was lower when older plants were inoculated compared to younger plants. The degree of mature plant resistance was cultivar dependent and for cv. Bintje occurred when plants were at least 9 weeks old. However, the degree of mature plant resistance was never complete, with even old plants yielding a proportion of infected tubers. Beemster (1958) suggested that old leaves were readily infected by inoculation, but that the vascular system became 'less penetrable for the virus' with age, leading to poor translocation of virus from foliage to tubers in older plants. Beemster (1969) also demonstrated non-uniform movement of PVX through mechanically inoculated potato plants. Inoculation of a leaf on one stem of a two-stemmed plant of cv. Bintje favoured transmission of PVX to tubers produced on the inoculated stem in comparison to those on the stem not inoculated.

Sources of inoculum

Volunteer potatoes may serve as a source of inoculum in climates with mild winters or where snow cover protects tubers from freezing (Franc and Banttari 2001). Wright and Bishop (1981) described a field with an estimated 30,300 volunteer potato stems per hectare, with 65% infection with PVX. Inadequate control of volunteer potato

prior to planting a potato crop would allow transmission of virus from volunteers to the crop during the growing season. Weeds may also act as a reservoir of inoculum into potato crops. Allen and Davis (1982) reported that PVX occurred in 11 of 28 weed species studied. Hairy nightshade (*Solanum sarachoides*) was found in six of seven fields examined and plants infected with PVX were found in five fields. PVX occurred in leaves, roots, fruits and seeds. Redroot pigweed (*Amaranthus retroflexus*) and Lambsquarter (*Chenopodium album*) were found in seven of seven and six of seven fields respectively and were shown to harbour PVX in five and three fields respectively. It was suggested that infected potato plants acted as an initial source of inoculum for weeds (Allen and Davis 1982). Such weeds could then act as a 'green bridge' for subsequent potato crops. Locatelli *et al.* (1978) detected PVX infection in 4/18 *Amaranthus powellii* and 7/14 hairy nightshade plants located in areas adjacent to potato fields in Oregon. Beemster (1977) reported that Bromegrass (*Bromus commutatus*) could be inoculated with PVX. Bromegrass developed symptoms of infection and PVX could be reisolated. However, only a small percentage of plants became infected and it is not known if bromegrass can be infected naturally and act as a reservoir of inoculum for potato crops.

2.3.5 Diagnostic host species

Indicator species such as *Nicotiana tabacum* and *Datura stramonium* infected with PVX provide diagnostic symptoms for strain type identification. PVX infections in tomato can cause slight stunting and mosaic. Symptoms in tobacco can distinguish minor variants in PVX strains. In tobacco, PVX may cause necrotic or mottle ring spotting (Bercks 1970). Ringspot symptoms are caused by many PVX isolates

infecting *N. tabacum*, while other strains cause systemic vein-clearing and mottling. At high temperatures some strains of PVX do not produce any symptoms (Bercks 1970; Brunt and Loebenstein 2001). In *D. stramonium* characteristic symptoms resulting from most PVX strains include systemic chlorotic rings, which may be preceded by mosaic and mottling depending on the PVX isolate used for inoculation (Bercks 1970; Brunt and Loebenstein 2001). A useful local lesion host is *Gomphrena globosa*, with the middle leaves (8-10 leaves) on the main shoot of the plant being the most suitable for mechanical inoculation (Bercks 1970). *G. globosa* is also a useful indicator plant for distinguishing PVX^{HB} from other PVX isolates, with PVX^{HB} being the only PVX strain that does not induce local lesions (Brunt and Loebenstein 2001). Other experimental hosts and symptoms include are listed (Table 2.8). Various *Nicotiana* spp. including *Nicotiana tabacum* provide useful propagation species (Bercks 1970).

Table 2.8. The reactions of five indicator hosts to infection by *Potato virus X* (PVX) (from Randles and Ogle 1997).

Indicator plant species	*Potato virus X*
Chenopodium amaranticolor	Local necrotic lesions
Datura stramonium	Local necrotic lesions
Gomphrena globosa	Local necrotic lesions
Nicotiana glutinosa	systemic mosaic and necrosis
Nicotiana tabacum	Local ringspot lesions, systemic ringspot and necrosis

2.3.6 Retention time of PVX in the absence of host plants

Stability and retention time of PVX in sap varies with different strains and various surfaces. The thermal inactivation point (10 min.) in tobacco sap depends on strain and ranges between 68 -76° C. The dilution end-point of PVX is between 10^{-5} and 10^{-6}. PVX retains infectious in sap for several weeks stored at 20°C and greater than one year in the presence of glycerol (Bercks 1970; Beemster and de Bokx 1987; Brunt and Loebenstein 2001). Wright (1974) demonstrated the retention time of PVX in potato leaf sap on various surfaces associated with potato production (Table 2.9).

Table 2.9. Retention time of infectivity of sap containing *Potato virus X* (PVX) determined by mechanical inoculation to Netted Gem potato or *Gomphrena globosa* L. on a range of surfaces related to commercial potato production (from Wright 1974).

Surface Type	Retention Infectivity Time
Leather	10 seconds
Unpainted wood, human skin, rubber and iron	3 hours
Painted wood, cotton and jute	6 hours
Soil	24 hours

2.3.7 Reinfection rates of PVS and PVX in the field

Franc and Banttari (2001) summarised much of the information of reinfection rates of PVX and PVS. In a study in Scotland, Cockerham (1958) demonstrated that PVX infection of seed potato of cv. Majestic planted in isolated plots to prevent

introduction of virus from outside sources, increased from approximately 1% to 4.8% over a 3 year period. In contrast, PVX incidence in plots of the same stock planted in plots surrounded by PVX infected potatoes increased to 82.9% over the same time-period. Cockerham (1958) suggested that the main means of transmission was from plant to plant contact between infected plants surrounding the plot and those within the plots. However, implements used in cultural operations were also implicated in virus transmission.

For some production areas, aphid transmissible isolates of PVS may influence reinfection rates (Bode and Weidemann 1971; Kostiw 1975; Wardrop *et al.* 1989). Reinfection of five potato cultivars derived from tissue culture and increased on four farms in Minnesota was monitored between 1976-1978 (Banttari *et al.* 1978). PVS was shown to reinfect stocks rapidly (Table 2.10) while reinfection with PVX and the aphid vectored PLRV was slight. Isolation from other potatoes and cultural practices were thought to be important factors influencing reintroduction of viruses.

Table 2.10. Reinfection of different cultivars of originally healthy potato stocks in Minnesota after 3 generations of field increase (from Banttari *et al.* 1978).

	Incidence of PVS and PVX (%)	
	PVS	PVX
Norland	24	1
Norchip	16	Trace
Kennebec	15	1
Norgold Russet	31	Trace
Russet Burbank	32	6

Differences in reinfection rates of PVS in cvs. Norgold Russet and Ontario and of PVX in LaChipper, Norchip and Norgold Russet with PVX were noted by Hahm *et al.* (1981), and attributed to the relative susceptibilities of cultivars to each virus and to differences in cultural practices between growers. For composite samples, 59% and 70% were infected with PVS and 19% and 55% were infected with PVX after one and three generations in the field respectively. Manzer *et al.* (1978) also reported that reinfection of seed stocks was more rapid with PVS than PVX.

Rapid reinfection of healthy potato stocks from point source inoculations with a non aphid-transmissible strain of PVS were reported by Franc and Banttari (1996). At two field sites over a two year period, reinfection rates of PVS for cv. Norland, and Russet Burbank were 71.8% and 73.0% respectively, both of which were significantly ($P<0.01$) higher than for Kennebec (29.5%). Khalil and Shalla (1982) showed transmission of a non aphid-transmissible strain of PVS to occur in an insect free greenhouse to 5/30 previously uninfected plants when in physical contact with infected plants for a period of 2 months. Similarly, in field plots, 11/108 plants became infected. de Bokx (1972) monitored the increase in PVS incidence in field plots established with approximately 10% of plants with secondary infections. After one growing season increased primary infections of 56-76% and 2-28% were noted for cvs. Eersteling and Alpha, respectively. The pattern of spread suggested that PVS was probably spread by foliar contact (de Bokx 1972). McDonald (1987) reported that PVS reinfection of initially virus-free material was significantly ($P\leq0.05$) faster when plantlets were transplanted into the field compared to the reinfection of plants propagated from tubers. Plantlets flowered 1-2 weeks later than plants derived from

tubers and McDonald (1987) considered that the faster onset of mature plant resistance in the latter material accounted for the difference in virus reinfection.
Rapid reinfection of Pathogen Tested potatoes with PVS and PVX was reported in New Zealand, with a mean incidence of 19% and 56% respectively for PVX and PVS within one season (Fletcher 1984).

Omer and El-Hassan (1992) demonstrated an increase in virus infection in the cultivar Alpha in the Sudan over time. In G0 crops imported as certified seed from Holland, G1 and G2, the incidence of PVS was 1.3%, 1.9% and 4.2% respectively and of PVX was 4.5%, 7.2% and 9.3% respectively. A local cultivar (Zalinge) grown for many years in Sudan had an incidence of PVS and PVX of 83.1% and 15.2%, respectively.

2.4 Detection methods for PVS and PVX

Slack and Singh (1998) summarised much of the literature on the historical development of methods for viral detection in potato. The more commonly used virus detection methods are outlined below and include indicator species, early serological techniques, enzyme-linked immunosorbent assay, electron microscopy, nucleic acid hybridisation, reverse transcriptase polymerase chain reaction and microarrays.

2.4.1 Indicator plants

The first report that tuber sap could be used to detect PVX was by Johnson (1925). In some early studies, large scale testing was done by abrading tubers on a coarse piece of sandpaper and rubbing the exposed surface onto indicator plants (e.g Bald and

White 1942). Mechanical inoculation of the host *Gomphrena globosa* and enumeration of the number of local lesions gave the opportunity to quantify PVX concentration. Using this method, high concentrations of PVX were found in tuber sprouts (Wilkinson and Blodgett 1948) and PVX was detected in various cultivars throughout the year (Hoyman 1951).

2.4.2 Early serological techniques

Serological testing of dormant tubers was pioneered by Gratia and Manil (1934) who demonstrated that PVX antiserum and sap from a PVX infected tuber produced a flocculation reaction. The technique was improved by Scott *et al.* (1964) by using bentonite to make the flocculation effect more visible. This flocculation technique was demonstrated to be more sensitive than microprecipitin tests (van Slogteren 1955) and was later extended to other potato viruses including PVS (Kahn *et al.* 1967). Broadscale use of serological tests to detect virus in North American seedlots was pioneered by Shepard and Claflin (1975) and Wright (1988). Initially, the serologically based Ouchterlony double-diffusion test was used (Shepard 1972). Summer testing of leaves for PVX was carried out in several States in North America, and seed marketed as 'PVX-tested' if below the threshold (Slack and Singh 1998). Agar diffusion tests have been used for detection and assessing strain relationship of PVX, although dissociation of PVX virions in soluble antigens is required to migrate into agar medium readily. Plant extract can be incubated with pyrrolidine (2.5% final concentration) to disassociate PVX (Shepard 1970 cited in Brunt and Loebenstein 2001).

The latex agglutination test (LAT) in which virus specific antibodies are adsorbed onto latex beads and mixed with sap has also been employed for the detection of potato viruses. This procedure is rapid (<15 minutes), but requires different concentrations of antigen as it is sensitive to virus concentration. For some potato viruses the LAT has been shown to be up to 1000 X more sensitive than microprecipitin tests (Koenig and Bode 1978). For elongated viruses such as PVS and PVX, the LAT was 100 x more sensitive than microprecipitin test, but in tuber samples the test underestimated the incidence of several viruses, including PVS and PVX (Fribourg and Nakashima 1984).

2.4.3 Enzyme-linked immunosorbent assay

The advent of enzyme linked-immunosorbent assay (ELISA) (Clark and Adams 1977; Voller *et al.* 1976) during the 1970's made feasible the large-scale application of serological testing for plant viruses. Attempts were made to detect viruses in dormant potato tubers, including PVX (de Bokx *et al.* 1980a) and PVS (de Bokx *et al.* 1980b). PVS was detected in tuber tissue by ELISA, but virus concentration declined slightly after 8 weeks of storage. No differences in detection were noted for PVS from tubers between bud and stem-end pieces or storage at 4°C or 20°C (de Bokx *et al.* 1980b). PVX could be detected in tubers stored at 4°C for 38-39 weeks, however detection was increased if tubers were removed from storage and held at 20°C for one week prior to testing (de Bokx *et al.* 1980a). Slack and Singh (1998) observed that while ELISA had been shown to detect both PVS and PVX in tubers immediately after harvest, it had not been as effective as testing foliage from growing plants during the season. Generally the best source of PVS for indexing in secondary infected potato

(PVS-infected plants resulting from planting of PVS-infected tubers) is within the middle leaves of the plant (Singh *et al.* 1989).

In tubers, a higher PVS titre has been reported in tuber sprouts compared to underlying tuber tissue (Kahn and Slack 1980; Franc and Banttari 1984). ELISA indexing is now routinely used by most seed certification schemes around the world, to supplement visual field inspections. For example, in the Netherlands some $5x10^6$ ELISA tests are conducted annually on seed potato (Huttinga 1996). A potentially more sensitive, faster and cheaper method for PVS testing than ELISA is direct tissue blotting (Samson 1993 cited in Brunt and Loebenstein 2001) or squash blot immunoassay (SBIA) (Mitchell *et al.* 1990). In this procedure, tuber tissues are squash-blotted onto a nylon membrane and treated with enzyme labelled specific antibodies and then enzyme substrate in a similar manner to the ELISA test. Potato viruses including PVX have been detected from tubers by SBIA (Slack and Singh 1998). In comparison to ELISA, SBIA is faster and labour saving for testing several potato viruses including PVX and PVS (Faccioli and Colombarni 1990 in Faccioli 2001). A similar protocol is routinely used in Western Australia to detect PLRV, with bunches of cut potato stem pieces blotted onto nitrocellulose membrane (Dr. R.A.C. Jones, DAFWA, *personal communication*).

A problem associated with ELISA is the limitation of detection levels. The inability to detect virus from infected leaf or tuber samples with low virus titer highlights the limitations of antibody-based techniques. Banttari and Franc (1982) showed PVS was only reliably assayed by ELISA in tubers with sprouts compared to tubers without sprouts.

2.4.4 Electron microscopy

Electron microscopy has proven to be a valuable tool in the diagnosis of viral infections. In many cases crude extracts from plants can be used and the presence of viral particles and their morphology can be determined within minutes. The availability of specific antibodies to particular viruses also allowed the development of immunosorbent electron microscopy in which grids are coated with specific antibodies to trap and concentrate virus particles and then visualised with an electron microscope. Electron microscopy remains a powerful tool for the visualisation of virus particles in samples from symptomatic plant tissue of uncertain disease etiology. For example, the recently described PotLV was found during routine electron microscope testing of accessions in the Vancouver collection of virus-free potatoes (Goth *et al.* 1999).

2.4.5 Nucleic acid hybridisation

With the development of methods to transcribe RNA to DNA in the laboratory (Taylor *et al.* 1976), nucleic acid hybridisation methods were developed for the detection of plant RNA viruses and viroids (e.g. Hull 1993). For potato, this technology has allowed the routine detection of *Potato spindle tuber viroid* (Family *Pospiviroidae*, genus *Pospiviroid*, PSTVd) (e.g. Owens and Diener 1981). Unlike viruses, viroids have no coat protein and are therefore unable to be detected by serological methods such as ELISA. Nucleic acid hybridisation techniques have also been used to detect and characterise strains of potato viruses (Slack and Singh 1998), including strains of PVS (Weidemann and Koenig 1990). Baulcombe and Fernandez-

Northcote (1988) used the nucleic acid spot hybridisation technique to detect PVY and PVX.

2.4.6 Reverse transcription polymerase chain reaction

The development of the PCR has signalled another breakthrough in the detection of viruses. By knowing the base sequence of parts of the target organism genome, specific short lengths of DNA complementary to this can be used as primers to multiply segments of the genome specific to particular viruses, to detectable levels. Reverse transcriptase polymerase chain reaction (RT-PCR) has been developed for viruses with RNA genomes, and initially involves the RNA strand to be reverse transcribed into complementary DNA (cDNA). Subsequently these cDNA molecules provide a template for PCR to amplify the specific DNA sequence (Barker *et al.* 1993).

Heldák (2001) used RT-PCR to detect PVS in potato plantlets grown *in vitro* and in regenerants from *in vitro* heat-treated shoot tips. The procedure detected PVS at a dilution of approximately $1:10^{-6}$, compared to between $1:10^{-3}$ to $1:10^{-4}$ for ELISA. ELISA tests are often unreliable in tissue culture material or such material soon after planting out due to low virus titre. A period of growth in the glasshouse is often required before testing becomes reliable. Although Barker *et al.* (1993) found ELISA to be more efficient, less expensive and less time-consuming than PCR, both PCR and ELISA were able to detect PVY in samples taken from tubers from growing plants and post harvest tubers stored for 3 weeks. However, PCR was unable to detect PVY in infected tubers stored for 20 weeks stored at 10°C. Although virus detection

declined for ELISA testing with only half of the positive tubers detected after being stored for 20 weeks, in this study the usually more sensitive technique of PCR was much less efficient than ELISA (Barker *et al.* 1993).

In a comparison between ELISA and RT-PCR, Heldák (2001) detected PVS in 65.6% and 71.9% of cuttings from regenerant plants respectively, indicating that RT-PCR was more sensitive at detecting PVS in tissue cultured material. RT-PCR can be affected by plant compounds such as polyphenolics, and protocols need to be carefully worked out to optimise tests (Singh and Nie 2003). Multiplex reverse transcription polymerase chain reaction (M-RT-PCR) is a variant of RT-PCR that allows the specific detection of multiple viruses or virus strains in one assay. Nie and Singh (2001) developed M-RT-PCR for the simultaneous detection of PVX, PVY, PLRV, PVA, PVS and PSTVd. This method was able to detect viruses in leaves, tubers and aphids. However, some viruses were detected more reliably in leaves than dormant tubers, and in some cultivars than others (Nie and Singh 2001). In addition Peiman and Xie (2006) developed M-RT-PCR for simultaneous detection of PVX, PVS and PLRV in potato leaves and dormant or stored tubers. Similarly M-RT-PCR was reliably able to detect PVX, PVS and PLRV in leaves and tubers, although virus detection was higher in leaves than in tubers. Peiman and Xie (2006) found M-RT-PCR method was more sensitive at detecting virus than ELISA in freshly harvested tubers, with a 3.2% increase in virus infections detected by M-RT-PCR compared with ELISA. M-RT-PCR can also be used to differentiate between strains of the same virus, for example strains of PVY (Singh and Nie 2003). Immunocapture PCR was modified for plant viruses by Wetzel *et al.* (1992), involving trapping and concentration of virus particles in a micro-centrifuge tube coated with specific

antibodies, followed by RT-PCR for amplification and detection by gel electrophoresis.

Real-time PCR is used to quantify a specific part of the given DNA during each cycle through PCR amplification reaction (Gachon *et al.* 2004). During each cycle of PCR, fluorochrome is incorporated into the new PCR product being synthesised and emits light that can be recorded. Thus, during the exponential phase of amplification real-time PCR, precise quantification of the amount of the starting material is possible in a sensitive, rapid and specific way (Gachon *et al.* 2004). Real-time PCR was used to detect RNA of PVY potato tuber necrotic strain (PVY^{NTN}) on non-inoculated leaves of different potato cultivars, during a study of potato cultivar susceptibility and sensitivity trials of PVY^{NTN}. Real-time PCR was shown to be more sensitive than other virus detection methods such as ELISA, immuno-serological electron microscopy and tissue printing (Mehle *et al.* 2004).

2.4.7 Microarrays

A more recent development of molecular detection techniques is microarray technology (e.g. Boonham *et al.* 2003). Microarrays or 'gene-chips' have been used to examine the expression levels of hundreds or thousands of genes simultaneously (Boonham *et al.* 2003). However, the technology is also applicable for the simultaneous detection of many plant pathogens. DNA sequences (probes) complementary to parts of the genomes of known pathogens are spotted or 'printed' onto a surface, such as a glass slide treated with poly-L-lysine (Bystřická *et al.* 2003). This procedure is often undertaken using robotically controlled equipment, which

allows miniaturisation of the process and the spotting of many hundreds or even thousands of such probes onto a single slide. Plant samples are subjected to an extraction procedure and resultant RNA/DNA is labelled with a biochemical fluorescent marker (usually Cy3-dUTP and Cy5-dUTP). The extracted plant sample is applied to the slide under controlled conditions, and if a particular pathogen is present in the sample its DNA/RNA will bind to the part of the slide containing the complimentary sequence. The slide is then washed and 'read' with sophisticated equipment (lazer and computer software system) to detect the fluorescent marker. Boonham *et al.* (2003) developed a microarray system that successfully detected several potato viruses (PVY, PVX, PVA, PVS^{O} and PVS^{A}) at the same limits of detection as ELISA. Similarly Bystřická *et al.* (2003) was able to detect PVA, PVS, PVX, PVY, *Potato mop top virus* (Family not assigned, genus *Pomovirus*, PMTV) and PLRV in potato samples using microarrays. Using this technology, it is possible that many potato pathogens could be screened in a single sample.

A similar approach is to use a protein array in which antibodies specific to particular proteins are printed onto the glass slide. It is feasible that such a system could be used in a similar manner to ELISA for the detection of viruses. At present, microarray technology is expensive. However, it has many beneficial features that might see it become an important methodology for mid to large-scale viral diagnostic testing in the future.

2.5 Effects of viruses on yield and quality of potato cultivars

Disease in a crop is a function of the three components of the 'disease triangle', which requires a susceptible host, conducive environmental conditions and a virulent pathogen (Ragsdale *et al.* 1994; Brown 1996). In addition to the three components of the typical plant disease triad, an insect-vector pathosystem requires a vector (the fourth variable) (Ragsdale *et al.* 1994) such as for PVS strains that are transmitted by aphids in a non-persistent manner (Slack 1981; Jones 1983). Cultural practises such as irrigation, fertilisation practices and soil and weed management can influence the incidence and severity of disease in potato and can have severe consequences on yield and the quality of the crop. In addition, climatic factors including temperature, humidity, water, intensity of light and day length can also influence disease incidence and severity (Rabinowitch and Levy 2001).

Historically the term 'running out' was used to describe the declining performance of potato cultivar over time. This phenomenon can now be attributed to mixed viral infection within the one plant and is common in plants such as potato that have been vegetatively propagated. The importance of individual effects of each virus on yield could not be identified until the availability of virus free stocks (Stace-Smith and Mellor 1968; Wright 1977). The first of the two viruses to be successfully eradicated from infected plants was PVX by a combination of heat treatment followed by propagation of axillary shoots from treated plants (Mellor and Stace-Smith 1967; Stace-Smith and Mellor 1968). The eradication of PVS proved more difficult than for PVX and also involved a combination of heat treatment followed by in vitro culture of

axillary buds excised from treated plants on a nutrient medium (Stace-Smith and Mellor 1968).

2.5.1 Effect of PVS on yield

PVS is generally considered to cause only mild yield reductions of potato. Of the few studies that have been conducted, many are dated and have shown inconsistent results. The economic importance of PVS is dependant upon virulence of isolate, incidence, environmental conditions and potato cultivar. Generally, yield from crops infected with PVS (most isolates) is reduced by 3-20% (Wetter 1971; Beukema and van der Zaag 1979; Brunt and Loebenstein 2001). Discrepancies exist between results of yield trials conducted on PVS infected potato crops and may possibly be attributed to differences in strain severity and potato cultivar. Tuber yield losses of up to 15% were reported from PVS infected potato crops not exhibiting chronic symptoms (Foster 1991). In a comparison of potato virus-free clones and several other PVS-infected clones the former produced 10% higher yield, attributed to a greater number of tubers (Kassanis 1965). In Europe, PVS has been reported to cause yield losses of up to 20% (Wetter 1970; Wright 1977). Single infections by PVS have been reported to reduce yield of potato in the Philippines by 8% (Luis 1981 cited in Hossain 1998). However, the PVS isolate utilised in a yield study by Wright (1977) showed no significant effect on yield of Russet Burbank (Netted Gem), except with co-infections of PVX (Wright 1977). There appear to have been no studies conducted on the effect of PVS^A in comparison to PVS^O in the literature, or the effect of PVS on potato in Australia.

2.5.2 Effect of PVX on yield

Since the discovery of PVX and associated degeneration of potato, many trials have been conducted on different cultivars to determine the effect of PVX on yield. Dowley (1973) summarised much of the early work. Mild strains of PVX had no effect or in some cases were associated with a minor increase in yield (Dowley 1973). For example, Rowberry and Johnson (1975) found no difference in yield of cv. Sebago between PVX-free, foundation or virus free lines in each of three years. However, more virulent strains of PVX were associated with yield losses between 2-47.9%, with large differences noted in the response of different cultivars (Dowley 1973). In addition, Emilsson and Gustafsson (1956) reported infection with PVX reduced dry matter content of tubers. In the cultivar Kerr's Pink, Dowley (1973) demonstrated that primary infection (healthy plants become infected within the season) with PVX had no effect on marketable yield (>6 cm diameter tubers). Conversely, secondary PVX infection significantly ($P<0.05$) reduced yield in comparison to healthy crops (45.2 t/ha vs. 47.9 t/ha). Primary infection had no effect on tuber size, but secondary infection (infected plants as a result of planting infected tubers) increased the percentage of marketable yield in the larger size by 15%. Percentage dry matter in tubers was reduced slightly by primary infection in comparison to 'virus-free' material (24.7% vs. 25.3%), but was unaffected by secondary infection. The dry matter yield/ha for primary infected PVX crops was significantly ($P<0.05$) lower than for healthy crops (11.0 t/ha and 11.5 t/ha respectively). Similarly, Dowley (1973) observed the yield of secondary infected PVX crops was significantly ($P<0.01$) lower than healthy crops (11.5 t/ha and 12.4 t/ha respectively). Teri *et al.* (1977) found no significant differences in total tuber yield for three cultivars infected with a mild strain

of PVX at Ithaca and Mount Pleasant, NY. However, at Riverhead, NY, the yield of larger tubers was significantly ($P<0.05$) reduced for all three cultivars. Tolerance of PVX varies between cultivars. For example cvs. Bake-King and Katahdin appeared less tolerant to a mild strain of PVX than cv. Hudson, although the statistical significance of interaction between virus and cultivar was reported to be low (Teri *et al.* 1977; Manzer *et al.* 1978). Although infections of latent PVX is not reported to effect yield of Russet Burbank (Netted Gem), mottle PVX alone affected yield and tuber size (Wright 1977). In the Philippines single infections by PVX have been reported to reduce yield of potato by 3.6-13.0 % (Luis 1981 cited in Hossian *et. al.* 1998).

Clones free of PVX and PVA produced 60% higher yield than infected clones (Gregorini and Lorenzi 1974). For some potato cultivars, crop losses due to a necrotic strain of PVX were around 50% and those due to PVS around 10-25% (Beemster and Rozendaal 1972).

2.5.3 Effect of coinfections of PVS with PVX on yield

Co-infections of PVS and PVX have been shown to have detrimental effects on yield of potato over and above that observed with single infections of either virus. Wright (1970) freed seed stocks of several cultivars by heat therapy and excision of axillary buds. He demonstrated that 'virus-free' seed stocks of Netted Gem (Russet Burbank) planted in British Columbia, Washington, Oregon and California yielded 11.6%, 8.6%, 15.6% and 26.1% more than seed lots from the same infected clones containing PVS and PVX. Differences were significantly different ($P=0.05$) at all sites except

Washington. Similarly 'virus-free' seed of cultivar White Rose planted in British Columbia, Washington, Oregon and California yielded 10.8%, -2.3%, 38.0% and 30.0% more than seed from the same clone containing PVS and PVX. Again, differences were statistically significantly different at all sites except Washington. In Oregon and California, 'virus-free' Netted Gem plants produced 21.2% and 31.3% more tubers per plant, respectively than infected plants and 'virus-free' White Rose plants produced 15.7% and 24.3% more tubers per plant respectively than infected plants. In a further field trial in British Columbia, healthy Netted Gem produced 10-12% more tubers and yields exceeded those of regular foundation seed stock by 11,652 kg/ha, with an increase in marketable yield of almost 30%. Wright (1977) demonstrated that Netted Gem with mixed infection of latent PVX and PVS in comparison to 'virus-free' plants produced significantly less total yield per hectare (36.1 t/ha vs 40.6 t/ha), significantly less marketable yield (24.2 t/ha vs 32.6 t/ha) and significantly fewer marketable tubers per plant (4.9 vs 6.3 tubers/plant). There was no significant difference in total number of tubers/plant or in specific gravity of tubers (Wright 1977). Manzer *et al.* (1978) reported that yield of healthy seed stocks of cultivars Kathadin, Kennebec and Russet Burbank were 3% , 5% and 10% greater than stocks infected with PVS alone, stocks co-infected with PVS and a mild strain of PVX or stocks co-infected with PVS and a moderate strain of PVX respectively. Infection was latent, with mosaic symptoms only observed in the latter case.

An earlier study by Wright (1970) found yield generally increased when potatoes were freed from PVX where co-infection occurred with PVS. However a study by Rowberry and Johnston (1975) suggested environmental factors may be involved in regulating the response to virus infection.

2.5.4 Effect of coinfections of PVX with PVY or PVA on yield

Combined infections of PVY or PVA with PVX may cause serious disease complexes in potato (Beemster and de Bokx 1987). Transmission of PVY occurs in a non-persistent manner by over 25 species of aphids and also via infected seed tubers (Agrios 1997). Mixed infections of PVX with PVY can result in a disease called rugose mosaic, associated with a reduction in tuber numbers and dwarfed plants (Agrios 1997). Yield losses of up to 50% have been reported for co-infections of PVX and PVY (Brunt and Loebenstein 2001). Mosaic symptoms of PVY in infected Shepody plants appear to be readily expressed in plants that possess co-infections with PVX. Typically these symptoms were exacerbated under conditions of low light (Draper *et al.* 2002).

Mixed infections of PVX with one of several other potato viruses have been reported to stimulate the multiplication of PVX (Rochow and Ross 1955; Stouffer and Ross 1961). For example synthesis of PVX may be markedly stimulated by coinfections with PVY in a host plant when, 1) PVY introduction is not by direct inoculation but introduction is by systemic movement, 2) the introduction of PVY occurs at a crucial stage of leaf development, 3) PVX is present during the rapid multiplication phase of PVY (Stouffer and Ross 1961).

Combinations of PVX with PVA can also result in significant yield losses, and symptoms include crinkling of leaves and severe mosaic (Stevenson *et al.* 2001). Plants grown from secondarily infected PLRV tubers may produce yield losses of up

to 33-50%. However, increased losses have been reported in crops where PVX co-occurs with PLRV (Brunt and Loebenstein 2001).

2.5.5 Effect of PVX on development of late blight (*Phytophthora infestans*) and other pathogens

Some studies have investigated the interaction between viruses and other pathogens of potato. Hossain *et al.* (1998) reported that inoculation of potato with PVX prior to inoculation with *Phytophthora infestans*, delayed the onset and reduced the severity of late blight. Prior inoculation with PVX reduced zoospore penetration, sporulation, size of lesions and increased peroxidase activity and yield in comparison to potato inoculated with *P. infestans* alone (Hossain *et al.* 1998). Hossain *et al.* (1998) suggested that PVX might have reduced foliar growth which in turn may have caused microclimatic changes around the plant resulting in depressed development of *P. infestans* (Hossain *et al.* 1998). Alternatively, low susceptibility of potato plants infected with PVX to *P. infestans* may be attributed to reduction or production of particular metabolites (Blumer *et al.* 1955 cited in Hossain *et al.* 1998). In addition, penetration of zoospores may be inhibited by nutritional changes of the host (Muller and Munro 1951 cited in Hossain *et al.* 1998). Hossain *et al.* (1998) suggested that breeders and pathologists should consider pathogen interactions either between pathogens or individual pathogen reactions when developing potato varieties. Conversely, Dowley (1973) demonstrated that primary infection with PVX increased the amount of tuber blight caused by *P. infestans*, with 3.0 t/ha of affected tubers from PVX infected potato and 2.2 t/ha from healthy material ($P \leq 0.05$). Dowley (1973) suggested that earlier maturity in PVX-infected plants leading to greater susceptibility

of foliar growth at an earlier stage might have explained the increase. Jones and Mullin (1974) reported that PVX-infected tubers were less susceptible to *Fusarium roseum* (dry rot) than uninfected tubers. However, many reports have shown that virus infection can increase the susceptibility of plants to fungal disease (e.g. Brunt 1986).

2.6 Seed certification schemes

2.6.1 Introduction

Tasmania has been presumed to be relatively potato virus free for several decades until recently. Several factors can be attributed to the apparent low incidence of virus, including the natural barrier of the Bass Strait and the presence of the Tasmanian Certified Seed Potato Scheme, which was introduced during the 1920s (Kirkwood 2003a). As previously mentioned PVS was originally detected in Tasmania in 1968 (P. Cross, DPIW, *personal communication*) and PVX-like symptoms were described 1929 (Oldaker 1929).

The advent of seed potato certification programs has been a fundamental management tool for the control of tuber borne pathogens since the early 20th Century. Many potato producing countries including many countries in Western Europe, USA and Canada (de Boer *et al.* 1996), New Zealand (Fletcher 1984) and Australia (Anon. 2001) utilise certification schemes for the production of seed potatoes. Such schemes ensure

quality of seed, that seed is of the correct cultivar and indicate the incidence of pathogens/disease.

Potato is normally propagated vegetatively, with tubers planted either whole or as seed pieces produced by cutting tubers into seed pieces. Vegetative propagation can result in problems in maintaining cultivar purity and management of 'seed-borne' diseases, with pathogens present in the propagating material transmitted to progeny tubers or transmitted between tubers as they are cut into seed pieces. Furthermore the cut surface of the tuber presents a large wound that can allow entry of pathogens from external sources.

Certification schemes developed from a recognition in the late 1800's that the performance of potato cultivars declined following several years of propagation, a condition known as 'degeneration' or 'running-out'. A disease referred to as leaf curl, probably caused by PLRV, was reported to cause epidemics as early as 1784 or 1812, more than a century earlier than the identification of PLRV (Bagnall 1988b). By the late 19th Century, it became recognised that symptoms of degeneration such as leaf curling, rolling, crinkling and blotching could be transmitted from plant to plant and from generation to generation through tubers (Slack 1993). Selection of progeny tubers from vigorous plants in the population for replanting was found to prevent degeneration. A system for inspection and production of potato seed stocks was first established in Germany, and formal seed certification schemes adopted in parts of the USA and Canada in 1913 (Slack and Singh 1998). The basis of certification schemes involved saving tubers only from those individual plants or 'hills' that appeared to be visually free of disease for replanting. These hill selections were often planted

together as a 'tuber-unit' in which a tuber from a hill was cut into seed pieces and planted sequentially in a unit. This unit was followed by the remainder of the tubers from the hill, which were also planted as units. If a disease appeared in any plant of a tuber-unit during the growing season, the entire unit was destroyed. This procedure was used for several decades as a means of producing and multiplying healthy seed stocks and is still used by many schemes for early generation material. Tuber-unit planting minimised inoculum sources for vectors during the growing season and the incidence of virus-infected tubers for the subsequent crop (Slack and Singh 1998). However, this system failed to control those pathogens that were latent and were consequently not detected during visual inspection. 'Tuber-indexing' was introduced in 1921 (Blodgett and Fernow 1921) in which an eye from the mother tuber was planted in the greenhouse during winter months. If virus symptoms were observed, the mother tuber was removed from the planting stock (Slack and Singh 1998). The tuber indexing method eliminated primary virus sources and therefore minimised virus spread by vectors. However, it potentially missed infected tubers when not all eyes were infected and furthermore required considerable space for growing plants (Slack and Singh 1998). Eventually, more stringent laboratory testing procedures and multiplication procedures were developed to ensure starting material was pathogen free. Seed certification schemes for potato have been adopted in many countries around the world, to ensure cultivar purity and 'trueness to type' and for the management of seed borne pathogens. Such schemes became useful for management of virus infections when these became recognised as the cause of degeneration. Seed certification has changed considerably over recent years with the development of laboratory and greenhouse techniques for the rapid multiplication of seed stocks and the development of rapid and sensitive laboratory tests for pathogens.

The main components of Seed Certification Schemes as outlined by McMorran and Moseley (2003) are the production of pathogen free stock and the limiting of generations over which a seed line can be vegetatively propagated.

Production of pathogen free stock

The production of healthy seed potato stock was revolutionised by the advent of the tissue culture techniques. Tissue culture enabled multiplication of selected seed stock under sterile laboratory conditions. The combination of meristematic tip culture with other treatments to free the propagule of virus is thought to occur either through an absence of virus in the meristematic region or, for some viruses, a loss of virus during *in vitro* culture. In tissue culture there is often a direct relationship between the size of the meristematic region used for tissue culture and the proportion of meristems that successfully develop into plantlets. However, there is often an inverse relationship between the size of the meristem and the proportion of resulting plantlets that are free of detectable virus. Various authors have shown that meristem tip culture alone is not sufficient to eradicate PVS from potato (Stace-Smith and Mellor 1968, Cassells and Long 1982, Klein and Livingston 1983). Excising meristems containing two leaf primordia led to 100% of regenerated plantlets being free of detectable PLRV, PVY and PVM, but one-third still contained PVX and PVS (Accatino 1966 cited in Faccioli and Marani 1998). Culturing smaller meristems (0.1 mm) with or without leaf primordia let to 95% of plantlets having no detectable PVS or PVX. However, only 10% of meristems developed into plantlets (Kassanis and Varma 1967). PVA and PVY were easily eliminated from 85-90% of meristems smaller than 0.3 mm long, while other viruses such as PVS and PVX were not eliminated (Morel *et al.* 1968). PLRV and PVY were completely eliminated by using meristems with four leaf

primordia (Faccioli and Rubies-Autonell 1982; Faccioli *et al.* 1988). PVS was shown to be present in the meristematic dome, both by electron microscopy (Zaklukiewicz 1983 cited in Facciol and Colombarini 1996) and by autoradiography (Rubies-Autonell *et al.* 1987, 1989). PVX has also been shown to occur in the meristematic region (Appiano and Pennazio 1972), whereas PVY and PLRV occur infrequently in this region (Faccioli and Rubies-Autonell 1982; Faccioli *et al.* 1988). Faccioli and Colombarini (1996) excised meristems from PVS infected plants with two leaf primordia (0.3-0.4 mm length) and were unable to detect virus in 38.2% of meristems. Some 34.7% of meristems successfully produced plantlets *in vitro*, of which 40% were virus free. In comparison only 10% of meristems containing four leaf primordia (0.5-0.8 mm length) had no detectable virus. Each of these developed an average of two sprouts and 81.7% successfully developed into plantlets, of which 14.3% had no detectable virus. Heat therapy prior to meristem tip culture has been used to free several potato cultivars of PVS and PVX (MacDonald 1973; Stace-Smith and Mellor 1968). Faccioli and Colombarini (1996) used heat therapy (35°C±1°C) of mother plants for 26, 33 or 37 days prior to excision of meristems with four leaf primordia. Between 57 and 62% of meristems successfully developing into plantlets, of which between 91% and 96% had no detectable virus. By comparison some 62% of meristems removed from mother plants exposed to 21°C±1°C successfully developed into plantlets, of which only 12% were free of detectable virus.

Chemotherapy, which utilises antiviral chemicals in association with meristem tip culture, has been successfully used for the eradication of several potato viruses (PVX, PVY, PVS and PVM) (Cassells 1987; Cassells and Long 1980; Cassells and Long 1982; Klein and Livingston 1982, 1983). Faccioli and Colombarini (1996) utilised

the antiviral chemicals ribavirin (Virazole) and DHT (2,4-dioxo-hexahydro-1,3,5-triazine) together at 5 mg/ml to improve the percentage of healthy plantlets. Ribavirin and DHT were applied either in the tissue culture medium or by soaking apices from stem cuttings for 15 days prior to meristematic excision in a Hoagland's modified solution containing these chemicals. For example in one case, 90% of treated meristems developed into plantlets of which 74% had no detectable PVS. By comparison, 90% of untreated meristems developed into plantlets, of which 42% had no detectable virus (Faccioli and Colombarini 1996).

The combined use of heat therapy and chemotherapy on *in vitro* plantlets enables virus free plants to be obtained from modified nodal cuttings rather than smaller meristem tips. This allows the regeneration of plants to be accomplished in 4-6 weeks rather than 3-6 months (Sanchez *et al.* 1991).

Currently, most tissue culture techniques involve the removal of the apical meristem and 1-3 leaf primordia (0.1-0.5 mm) from a tuber sprout or stem of a potato plant. The meristem is grown on defined media *in vitro* to produce a plantlet. Nodal cuttings are taken from the plantlet and consist of a stem segment containing an axillary growing point and a leaf. These are further grown on sterile media. The process is repeated several times to produce sufficient plantlets for further increase in the form of minituber production (described below). Alternatively, cuttings can be further cultured *in vitro* to produce microtubers (Donnelly *et al.* 2003) which are then used for minituber production. Propagules produced *in vitro* (microtubers or plantlets) are then planted in beds and grown in protected and controlled environments at high density to produce minitubers (Watad *et al.* 2001), approximately 1.3 to 5.1 cm in

size. A high standard of hygiene is used, along with rigorous inspection and testing to prevent reinfection of plants.

During the initial nodal cutting process, pieces of plantlet are tested for pathogens. For example in the certification scheme in Oregon, USA, plantlets are tested for bacterial soft rot, blackleg, ring rot, PSTVd, PLRV, PVY, PVX, PVS, PVA and PVM (McMorran and Mosley 2003). For tissue culture stock, 100% of plantlets are tested. During the latter stages of seed stock development a representative sample comprising some 0.5-25% of plants is pathogen tested. Several months after planting, minitubers are harvested and stored until the following growing season. Minitubers or tissue culture plantlets that are planted in the field are the initial source of certified seed potato lots. These lots are then multiplied and increased further for commercial use. During this process, plants are subject to regular inspection and pathogen testing.

Limited generations

During the production of seed or commercial potato, the plant is continually exposed to infection by pathogens. The probability of a seed lot becoming infected increases with every year the lot is in field production. To minimise this risk, the majority of seed certification agencies restrict the number of years that a seed lot can be eligible for certification. This is known as limited generation and refers to the number of years that the seed lot can be increased in the field following release of material from the greenhouse. There is therefore a 'flushing-out' of the system whereby healthy material is continually introduced to the scheme. In the USA, the generations are limited to five to nine years, depending on the seed production area.

Individual seed certification agencies also have their own specific tolerances for particular diseases and other regulations for each successive year the seed lot is in the field. In Canada, seed certification is under the control of the Federal Government. However, in the USA, seed potato certification is the responsibility of either a land-grant University, a State Department of Agriculture or a grower (Crop Improvement) association. This leads to a diversity of rules and regulations between particular schemes.

The following overview of the Tasmanian seed certification schemes is given mainly in relation to virus diseases. Note that certification schemes also contain tolerance levels for other factors including bacterial diseases and purity of cultivar that are not included in the following review.

2.6.2 Tasmanian seed scheme and Australian National Standard for Certification of Seed Potato

History of seed potato in Tasmania

In the early 1920's growers in Tasmania were advised by the Department of Agriculture to use seed of known history and to save seed from the healthiest and best growing plants (Taylor 2003). Progressive growers maintained their own seed plots. These were inspected during the season and any unhealthy plants, or those that were weak or not true to type were removed. Yield reductions due to virus diseases were one of the main reasons for the start of the certified seed schemes in the 1930's. In 1928, Department of Agriculture officers collected healthy, true-to-type plants from highland regions to use as foundation stock in what was to become the Tasmanian

Seed Potato Certification Scheme (Taylor 2003). Seed was planted at Myrtle Bank in the north-east of the State at an altitude of 580 metres, to avoid potential virus transmission by aphid vectors. Selections were assessed for quality, disease, vigour and the absence of virus symptoms. The trial was repeated in 1929, and included cultivars imported from UK and USA. In 1930, seed stocks were transferred to Hampshire in the north-west to produce disease free seed. Seed potato was grown on in Upper Natone, and in 1933, seed was distributed to 19 farmers under a certification scheme. The harvest from this crop was then certified by the Department of Agriculture. Tewkesbury Potato Station in the north-west was established in 1933 to raise mother seed for the Tasmanian industry, and by 1935 consisted of 33 acres, six cultivars and 1000 individual plant selections (Taylor 2003). During the 1960's a virus free line of Russet Burbank was imported from Canada and included in the collection of pathogen free cultivars developed by the Department. During the 1960's heat therapy was used to ensure mother plants were free of virus and tissue culture used to bulk up foundation stock. Plants were further bulked by taking stem cuttings and grown on under mist. The resultant tubers were then planted at Tewkesbury Potato Station. Seed produced in this way became available during the 1970's (Taylor 2003). A mini-tuber production facility was established at the Department of Primary Industries offices in Devonport in 1988. The use of mini-tubers and a move by seed growers to produce all generations of planting stock reduced the need for the Tewkesbury potato station and this was sold in 2000. In the mid-1990s, there was a relaxation in the altitude requirement for seed potato from 220 m to 180 m, then less than 180 m with ELISA testing for PLRV. Low incidence of PLRV continued to be observed in Tasmanian crops so the requirement for ELISA testing was abolished. This has led to a situation where seed potato crops are now grown in regions also used

for the production of ware crops and in proximity to urban areas with non-certified potato in gardens. Despite the lifting of altitude restrictions, PLRV in Tasmanian crops has remained at a low level in recent years. Both PLRV and some strains of PVS are transmitted by the aphid vector *M. persicae*, and this low levels of PLRV in Tasmania suggesting that aphid activity is currently not a major factor in virus spread in Tasmania. Therefore the apparent increase in PVS is likely to have arisen from other factors. The growing of seed crops in close proximity to ware crops is likely to have increased the potential for mechanical transmission into early generation seed. Control of volunteer potato is a major issue for the Tasmanian industry and there is potential for virus to survive between periods of rotation in this manner.

Tasmanian plant disease records state, PVS was first recorded in Tasmania in 1968, (P. Cross, DPIW, personal *communication*) and PVX-like symptoms were reported in Tasmania in 1929 (Oldaker 1929). Reports indicate diseased plants were replaced with clean stock, however, eradication of these viruses in Tasmania was unlikely due to a number of reasons. Over the years sporadic virus testing conducted in Tasmania found low levels of PVS, PVX and PLRV (P. Cross, DPIW, *personal communication*). Another factor that may result in a potential increase in virus status in Tasmania over time include a change to contract harvesting and centralised grading, seed cutting and cool storage in recent years. Seed cutting has changed from hand cutting to the use of cup cutters to the adoption in the last 20 years of mechanised, large volume cutters. A large proportion of the crop in Tasmania is cut at four centralised facilities, although hand cutting is still utilised for early generation crops.

Tasmania and other States had Seed Certification Standards for many years. However, in 2001 the States agreed on a National Standard (Anon. 2001) (outlined below).

National Standard for Certification of Seed Potato

Seed potato certification in Australia, is governed by the National Standard for Certification of Seed Potato. The National Standard replaced five separate State-based standards with a uniform minimum standard for disease, trueness to type and defects. The standard is overseen by the Australian Potato Industry Council (APIC) and is a voluntary scheme.

The initiation of a national compliance has transformed traditional seed certification schemes in many states. Under the National Standard, all potato stocks for use as starting material in the Certification Scheme must be visually free of diseases before being pathogen tested by an approved laboratory for over 20 pathogens including the viruses PLRV, PVA, PVM, PVS, PVX, PVY, TSWV, AMV and viroid PSTVd. This pre-nuclear material is maintained at the Institute for Horticultural Development, Knoxfield, Victoria or at the Tasmanian Department of Primary Industries and Water (DPIW). Pathogen tested stocks are then maintained *in vitro* to minimise the risk of reinfection and re-tested prior to their release to accredited facilities for further multiplication. Accredited facilities produce G0 seed, e.g. as minitubers, microtubers or plantlets. In the case of mintubers, leaf samples are collected for virus testing by ELISA. Samples are collected by approved agents after a growth period of 12 weeks and prior to senescence, and consist of one mature leaflet from every 20^{th} plant. Further bulking up of the seed lot in the field occurs from G1 to G5. Although G5 is

accepted under the National Standard, in Tasmanian multiplication of G5 for seed is generally not permitted, except under exceptional circumstances (L. White, DPIW, *personal communication*). There is also a requirement for rotations and separation distances between generations (Table 2.11) (Anon. 2001).

Fields are inspected two times during the season, with the first inspection around row closure and the second close to, but before crop maturation or just prior to top removal. For crops less than 4 ha the recommended sample size is at the discretion of the Certifying Officer. For crops greater than 4 ha, 500 plants/ha are inspected with a minimum of 2000 plants, both along rows and a traverse across the crop. Allowable levels of rogueing are at the discretion of the certifying authority, however as a guide a maximum rogueing level of 1% above the defined tolerance should be achievable in a certified crop. Where only part of a crop is deemed certifiable, rejected plants must be removed prior to harvest of the remaining crop (Anon. 2001).

Table 2.11 Required rotations and separation distances for crops to be eligible for certification under the Australian National Standard for certification of seed potato.

	G0	G1	G2	G3	G4	G5
Rotation [a]	Laboratory/ greenhouse	5 years	5 years	5 years	3 years	3 years
Isolation from commercial crop planted with certified seed [b]	N/a	20 m	20 m	20 m	20 m	1 blank row
Isolation from commercial crop not planted with certified seed	N/a	20 m	20 m	20 m	20 m	20 m

[a] As for other schemes around the world, fields selected for seed potato must meet further criteria regarding risk of bacterial wilt, presence of potato cyst nematode, etc.

[b] In addition, a minimum of 1 blank row between G1-G4 crops and between G4 and G5, with a minimum of 5 m between G5 and G1-G3.

All generations are given a rating of 1 to 3 according to specified tolerances for foreign cultivars, virus diseases, other diseases and total diseased plants (Table 2.12). Any seed with a field rating of 3 cannot be further multiplied for certified seed. However, in exceptional circumstances the Standard allows a lot with a rating of 3 to be multiplied for one further generation. Any generation of seed intended for multiplication in the scheme must be of a rating equal to or higher than that of the next generation (e.g. seed with a rating of 2 cannot be upgraded to 1 in the subsequent generation). Furthermore, the highest rating in any category (e.g. foreign cultivars, virus, total diseased plants etc.) determines the overall rating for the crop. For example a crop with a foreign cultivar rating of 1, virus rating of 2 and rating for other diseases of 3 will be given an overall rating of 3 (Anon. 2001).

Table 2.12. Rating system used for all generations with respect to incidence (%) of virus symptoms at each inspection. (note that other tolerances apply for foreign cultivars and other diseases).

	Rating 1		**Rating 2**		**Rating 3**	
Inspection:	First	Second	First	Second	First	Second
Virus diseases[a]	0.10	0.01	0.25	0.10	1.00	1.00

[a] Note there is a zero tolerance for potato cyst nematode, bacterial wilt (*Ralstonia solanacearum*) and potato spindle tuber viroid.

Visual inspections are also carried out on tubers following harvest. For lots <10 tonnes, 10-20 tonnes, 20-30 tonnes and 30-60 tonnes the number of samples to be inspected is 2 or 3, 3 or more, 4 or more, and 5 or more respectively. Samples consist of 200 tubers from bulk containers or all seed in a bag for bagged seed. These are visually inspected for various diseases, nematode, insect damage and defects. A maximum tolerance of 2% is set for all diseases and 2% for insect damage/tuber defects. For lots of less than 10 tonnes all samples above must pass, for 10-30 tonnes one borderline sample is acceptable and for 30 to 60 tonnes two borderline samples are acceptable. Seed which meets these tolerances will be graded 'A'. Seed from G1 to G5 can be sold as Certified seed if it has a tuber rating of 'A' and a minimum field rating of 3 and will be sold as 'Certified A' stamped with the generation (Anon. 2001).

Seed must be stored under conditions approved by inspectors of the certifying authority. During storage, each generation of seed must be separated to prevent mixing of lines, seed potato must be separated from ware potato and seed must be

clearly labelled. Seed must be packed and transported in new sacks or bins or in used bins and bulk trucks if accompanied by a cleanliness declaration certificate (Anon. 2001).

The National Standard also makes recommendations with regard to hygiene and requires that growers and Certification Officers ensure that a level of hygiene is adopted which will facilitate the production of high quality certified seed. Access to seed crops is to be limited to personnel authorised by the grower. All agronomic operations on seed crops should be conducted in order of health status, e.g. G2 before G3 etc., or hygiene precautions should be implemented between crops (e.g. wash down of machinery). Travelling irrigators should not be used where they would pass from seed crops sown with G4-G5 to crops sown with G1/G2 seed unless the irrigator laneway is sufficiently wide to allow the irrigator to pass through without making contact with the crop. The headlands normally used for machinery movement must not be planted and must be kept free of weeds. Soil and crop debris should not be allowed to accumulate in sheds. Waste potatoes, soil and crop debris are to be regularly removed from the shed and surrounding areas and disposed of in a dedicated pit or waste disposal facility. Waste is not to be returned to potato paddocks. All sheds, machinery and containers used for seed storage should be washed between seasons, or more frequently as required. Machinery should be cleaned with an approved disinfectant/sterilant (Anon. 2001).

2.6.3 Differences between the Australian National Standard and other schemes

Most certification schemes in other countries have a sliding scale of tolerances for diseases from very low tolerance in early generations, increasing to slightly higher tolerances in later generations. While Western Australia has adopted a range of tolerances between generations, the Australian National Standard requires all generations to meet the same set of tolerances (Rating 3).

Most other schemes have different grades of certified seed, each with differing tolerances for diseases. The Australian National Standard has only one grading, although the potential for two grades of tuber quality in the future is raised in appendix 5 of the Australian National Standard.

Many other schemes involve tuber testing after harvest to detect late season virus infections. Normally this involves a grow-out test over winter and laboratory testing of sprouts/leaves for virus. For example, in the USA samples of 300-800 small seed (30-60 g) tubers per seed lot are collected, dormancy broken and generally planted in California and Florida where winter growing conditions favour expression of virus symptoms (Slack and Singh 1998). Seed lots are visually inspected around 60 days after emergence and evaluated for virus infection, cultivar mixtures and other seed related issues such as herbicide exposure that may affect the performance of the following crop. Virus incidence in winter testing is particularly important as late season flights of green peach aphids can transmit PLRV which would remain undetected until tubers were planted the following season. Tolerance of leafroll,

mosaic and spindle tuber cannot exceed a total of 0.5% in early generation or Foundation classes and 5% in Certified class. This is a higher tolerance than the summer inspection because the tuber sample is smaller and therefore prone to more sampling error. Under the Australian National Standard, only a visual inspection of tubers is carried out after harvest. This would detect only those virus infections that caused obvious external tuber symptoms.

Many other schemes involve mandatory laboratory testing (usually by ELISA) for latent viruses of laboratory/greenhouse material and early field generation material. The Australian Standard does not require laboratory testing of early field generation material.

Some schemes require that all potatoes grown by a single farming operation must be inspected. This encourages growing seed crops in isolation from ware crops and prevents contact of the certified seed crop with storage areas or equipment used for ware potato (Franc and Banttari 2001).

2.7 Control strategies to reduce virus infections in potato crops

A number of grower practices summarised by Fletcher (2000) provide strategies growers may adopt to reduce virus incidence in potato crops and the following is based on his recommendations.

2.7.1 General strategies

General strategies for management of viruses includes use of certified seed, crop rotations, (e.g. in New Zealand a recommended 5-7 years between seed crops and 3-5 years for ware crops) and monitoring of crops for latent viruses as part of certification, with laboratory testing of plants.

2.7.2 Strategies to reduce spread of mechanically transmitted viruses

Spread of mechanically transmitted viruses may be reduced by adopting a number of cultural practices including handle tubers carefully to avoid damage and ensuring that storage bins, storage areas and grading equipment are clean and sanitised. In addition ensuring seed is cut hygienically with disinfected knives is an important strategy to minimise virus transmission in seed potato. Hygienic practises including washing or steam cleaning knives or machine blades prior to disinfection to remove potato wastes that may inhibit the activity of disinfectants. In addition the use of a suitable disinfectant frequently between seedlines, at rest breaks and at the end of the day can reduce the risk of virus transmission. As virus concentration is often higher in developing sprouts, so avoiding cutting tubers with long sprouts and allow cut tubers to dry can reduce virus transmission. Ensure tubers are planted prior to the formation of long sprouts as virus concentration often increases rapidly in the developing sprout and long sprouts can be damaged easily during handling, facilitating virus transmission between infected and healthy tubers (Fletcher 2000).

Disinfectant practices for reducing mechanical transmission of virus and the most common procedures for treating surfaces for viruses are heat disinfestation or chemical disinfection. Dipping cutting knives into alcohol and flaming, or steam treatment can be effective treatments. For chemical disinfection, surfaces must first be washed free of debris to allow contact between disinfectant and virus and to reduce the possibility of inactivation of the sterilant by organic matter. Furthermore, sufficient contact time must be allowed between the chemical and the surface. Most studies on disinfectants in potato production have been in relation to the control of fungal and bacterial plant pathogens (e.g. de Boer 2002). Less literature is available for viruses, and growers should seek advice from manufacturers of particular disinfectants as to their efficacy against viruses. Disinfectants are mainly used to kill bacteria, but many also inactivate viruses. Suitable disinfectants include 0.01% a.i. sodium hypochlorite (bleach) or a similar strong alkaline detergent, as viruses tend to be inactivated under alkaline conditions of pH 8.5 or higher (Secor and Gudmestad 1993). Calcium hydroxide (slaked lime) and 3% trisodium phosphate (TSP) also inactivate viruses (Secor and Gudmestad 1993). Broadbent (1963) and Mulholland (1962) also reported TSP to inactivate viruses at 3% in water and 10% plus detergent, respectively. However, activity of a particular disinfectant may be virus/viroid specific. For example, Anon. (1997) reported citrus exocortis viroid was inactivated on budding knives with sodium hypochlorite at 0.26% a.i./one second, but *Tomato mosaic virus* (Family not assigned, genus *Tobamvirus*, ToMV) was not. A range of disinfectants are effective at controlling *Pepino mosaic virus* (Family *Flexiviridae*, genus *Potexvirus*, PepMV) including Menno Florades (Benzoic acid), Jet 5, Virkon® S, GluCid, Panicide M, Trisodium Phosphate (10%) and Horticide (Grodan 2002). For PSTVd, Anon. (2002b) suggested tools could be effectively disinfected with fresh

solutions of sodium or calcium hypochloride (bleach) at 0.25% a.i. or greater and 2% sodium hydroxide. In addition chemicals such as Virkon® S and quaternary ammonium compounds such as Dermasan could be used. Stijger (1996) treated knives infested with *Pepper mild mottle virus* (Family not assigned, genus *Tobamovirus*, PMMV) with 1, 2 or 5% Virkon® S for 1 minute and reduced transmission to indicator plants by 99.4%, 98.8% and 99.6% respectively compared to untreated knives. Fletcher *et al.* (2004) showed decontamination of PVX from scalpel blades with benzoic acid at 5%, sodium hypochloride at 0.24% a.i, Virkon at 1% and Chitosan at 0.01% and reduced transmission by 100%, 90%, 90% and 89.9%. Formalin, Phenols (Amphyl, Lysol, CM-19) and the quaternary ammonium compound (Physan-20) are reportedly not effective against viruses (Anon. 2003a).

To reduce virus transmission recommended practices include ensuring if possible the use of herbicides for weed control and avoid inter-row cultivation. For example, in some locations weeds are controlled mechanically before potato plants have emerged. However, if this operation is done after emergence there is the possibility of mechanical spread of PVY (Banttari 1994) and other mechanically transmissible viruses. In addition maintaining a clean seedbed, free of trash, weeds and volunteers is import to reduce the potential of virus source. Weed species of particular importance include *Solanum* spp. such as *Solanum nigrum* and potentially *Chenopodium* spp. (*C. album*). To reduce the risk of plant damage and plant contact with machinery, early moulding is essential. In addition it is essential that clean clothing be worn between seed crops to minimise the risk of virus transmission between crops, and movement from crops of highest health status to those of lower or unknown health status rather than vice versa is recommended (e.g. movement from a G1 to a G4 crop). It is

important to ensure that width of irrigation tracks is sufficient to prevent contact between machinery (e.g. travelling gun irrigator) and plants and crops are not sown in the irrigation track. Movement of people and machinery should be restricted through the crop after rows have begun to close over to minimise risk of virus transmission. During crop inspections it is important to rogue suspected virus-infected plants shortly after emergence. It is also important that potato trash is removed and buried, waste tubers fed to livestock and volunteer plants sprayed off with herbicide.

2.7.3 Strategies to reduce spread of aphid transmitted viruses

Those viruses that are transmitted by aphid species are generally transmitted in either a 'persistent' (e.g. PLRV) or 'non-persistent' manner (e.g. PVS, PVY). Non-persistently transmitted viruses are acquired quickly by the aphid and are immediately transmissible to another plant. The aphid loses its ability to transmit the virus after probing a succession of healthy plants, and only becomes viruliferous again if it feeds on an infected plant. In contrast, persistently transmitted viruses have a latent period of several hours/days between acquisition and the ability to transmit virus. However, once acquired, the aphid vector retains the ability to transmit the virus for the rest of its life. Strategies for the control of aphid transmitted viruses are listed below.

Spray off seed crops

In some situations seed crops can be sprayed off with a desiccant as soon as possible after final certification to avoid late season virus transmission by aphids. In New Brunswick, USA for example, a vine kill date is established after a cumulative total of five green peach aphids have been caught in any one yellow pan trap. However, this

method is ineffective when there is early season aphid activity, which often occurs in other States such as Minnesota (Radcliffe *et al.* 1993).

Growing at altitude or in isolated areas away from aphid flights

Growing seed crops in areas isolated from aphid flights and potential sources of virus is often used in certification schemes as a means to control spread of aphid-borne viruses. No specific work has been conducted on isolation distances for PVS. However, some work had been done for PVY which is aphid transmitted in a non-persistent manner similar to PVS. The isolation distance may vary widely with the location. Hille Ris Lambers (1972 cited in Ragsdale *et al.* 2001) reported that viral epidemics could occur in the Netherlands when green peach aphids arrived from other locations 100-150 km away. In British Columbia, Canada, elite seed is grown in the Pemberton valley, 150 km from commercial potato crops (Ragsdale *et al.* 2001). However, in other locations the isolation distance has been shown to be less. In Eastern Idaho the isolation distance recommended for seed potato is from 400 m to 5 km from a known source of PVY, and up to 32 km from sources of PLRV (Halbert *et al.* 1999 cited in Ragsdale *et al.* 2001). The isolation distance recommended is due to the persist manner of aphid transmission of PVY and PLRV. In England, a distance of 800 m from PVY sources is recommended (Harrington *et al.* 1986). In Denmark, a distance of 40 m reduced spread of PVY (Hiddema 1972). Changed cropping practices in regions may also influence virus spread to seed crops and isolation distance. Ragsdale *et al.* (2001) reported that in areas where canola production has increased, there had sometimes been increased rejection of seed potato crops for virus. This was due to the canola providing a host for green peach aphid and other vector aphid species. Ragsdale *et al.* (2001) noted that areas where normal winter

temperatures fall below the limits of survival for green peach aphid are better for seed potato production. In such areas, aphid colonisation of the potato crop was delayed until later in the season, minimising the time the crop was exposed to inoculum and mature plants exhibiting greater resistance to infection. In some countries, seed production areas isolated from vectors are not available (e.g. Bangladesh, Italy, Philipines, South Africa, South Korea and Taiwan). In these countries the production of microtubers produced *in vitro* provide a means of bulking up initial seed material (Donnelly *et al.* 2003).

Other strategies for control of aphid transmitted viruses include:

i) Store seed in aphid proof stores – aphids can transmit virus to (and between) sprouted tubers in storage
ii) Weed control around margins to help eliminate aphid havens
iii) Sow in fields free of volunteer potato.
iv) Clean up cull piles which may act as sources of inoculum

Aphicides

Pesticide treatment of tubers before sowing with registered chemicals and pesticide application during the season can reduce infection with aphid borne viruses that are persistently transmitted (e.g. PLRV). This is especially the case where sources of PLRV are mainly infected plants within the crop and where winged aphids rarely arrive carrying PLRV, e.g. in the Red River Valley, Minnesota, USA (Hanafi *et al.* 1989). In this case, apterous (non-winged) aphids within the crop are responsible for most spread and there is sufficient time between acquisition and aphids becoming able to transmit the virus for the aphids to be killed by the systemic insecticide. However,

insecticide is relatively ineffective against non-persistently transmitted viruses such as PVS and PVY. DiFonzo *et al.* (1996 a,b) reported that control of the non-persistently transmitted PVY in early generation seed lots was not possible with insecticides in the Red River Valley as alatae aphids brought virus in from neighbouring crops and weeds and could transmit virus before insecticide took effect. Other studies have shown that the systemic insecticide imidacloprid can reduce PLRV, but had no effect on PVY (Boiteau and Singh 1999). The more recently released insecticide pymetrozine interferes with stylet penetration (Harrewijn and Kayser 1997) and is now widely used in the American potato industry. Many studies have shown mineral oils to reduce non-persistent transmission of viruses such as PVY (e.g. Boiteau and Singh 1982). However, oils can be phytotoxic, especially when mixed with fungicides or if applied when temperatures are high (Boiteau and Singh 1982). Adoption has been low because of these problems, and also due to the need for repeated application to offset the effects of weathering and emergence of new foliage.

Crop scouting for aphid vectors

Fletcher (2000) suggested that in New Zealand, crops be scouted for aphids soon after emergence. Potential hot-spots such as near gaps in windbreaks, paddock margins and gateways could be examined, working from the direction of the prevailing wind which may blow vectors in. During the season, a suggested sampling protocol was to examine the top, mid and base leaves of 50 plants selected randomly within the crop. If there were more than 10 wingless aphids/100 leaves it was likely that aphids were colonising and the crop should be sprayed with insecticide (Fletcher 2000). Thresholds for aphids in seed crops for control of PLRV have been determined overseas. For example in Minnesota, USA, Flanders *et al.* (1991) reported that PLRV

spread from a point source in plots of the PLRV susceptible cultivar Russet Burbank sprayed with methamidophos at 10 green peach aphids/100 leaves had no greater PLRV incidence than in plots treated with a systemic aphicide at planting followed by weekly sprays of methamidophos. Thresholds were fine-tuned for other cultivars with the threshold increased to 30 green peach aphids/100 leaves for cv. Kennebeck which is moderately resistant to PLRV and to 300 green peach aphids/100 leaves for cv. Cascade which is highly resistant to PLRV (DiFonzo *et al.* 1995). Thresholds have been determined for fresh market crops. For example, in south central Idaho, economic losses from PLRV net necrosis are predicted when the population density of wingless *M. persicae* exceeds 10 per 50 leaves for two consecutive weeks (Radcliffe *et al.* 1993). In Minnesota, North Dakota and Wisconsin the action threshold for fresh market production is 30 wingless aphids per 100 leaves while in California insecticide is recommended when 5% of the leaves are infested (Radcliffe *et al.* 1993). In New Brunswick, the action threshold is when 10% of the plants are infested with 25 aphids per plant. In Pennsylvania dynamic action thresholds based on accumulated day degrees have been used (Radcliffe *et al.* 1993). The relationship between aphid flights and incidence of PLRV in crops is well documented (e.g. Thomas 1997, Robert *et al.* 2000). However, in Canterbury New Zealand, Teulon and Stufkens (2001) found no relationship between aphid vectors caught in a suction trap over a 28 year period and the incidence of primary and secondary PLRV in seed potato. They proposed that factors such as the source of aphid immigrants (from infected weeds or non-infected hosts), the relative importance of alatae and apterous aphids in virus transmission, and vector control strategies employed over the years may have served to mask the relationship between aphid numbers and virus incidence. It was suggested that more complex models such as that of Nemecek *et al.* (1995) could be required to

understand the relationship between virus and vector. However, Teulon and Stufkens (2001) noted that the weekly provision of aphid flight activity data was well received by growers and used as an indication of virus risk in pest management.

Border crops for control of non-persistently aphid-borne viruses

Incoming flights of aphids tend to colonise the edges of fields where the contrast between green plants and dark soil is greatest (A'Brook 1968, A'Brook 1973, Smith 1969, Storey and Godwin 1953) and where eddies in wind currents deposit aphids (Broadbent *et al.* 1951, Johnson 1950). Producers of early generation seed potato often keep a cultivated soil border around the green seed crop that keeps the border free of weeds and potential hosts of virus, but also creates a crop/soil edge attractive to landing aphids that may be carrying PVY from outside the crop (Difonzo *et al.* 1996 a,b). Early generation seed fields are particularly vulnerable as they are usually small and have a large amount of edge relative to area. DiFonzo *et al.* (1996 a,b) found that PVY in tubers at the end of the season could be reduced from 47.8% in crops with cultivated borders to 35.0% in crops with a 12-15 foot border in 1992 and from 6.8% to 2.7% respectively in 1993. The most effective border was soybean as it was not a host for aphid vectors or potato viruses, however sorghum and wheat (which are hosts of grain aphids) also worked well (DiFonzo *et al.* 1996 a,b) as did a border of potato. In seed crops with a cultivated border, aphids tended to settle on the soil/potato interface and transmit virus into the potato crop. In crops surrounded by a crop border, aphids tended to settle at the soil/crop border and following feeding on the crop boarder lost their charge of virus (DiFonzo *et al.* 1996 a,b). Central to the use of border crops is to have a fallow area adjacent to the outside edge of the border crop, with no gap between the border and the potato crop. This maximises the chances of

aphids preferentially settling at the edge of the border crop and removing non-persistently transmitted virus as aphids sample the border plants. Seed certification practices do not commonly take advantage of border crops, with fallow ground or skip rows often used to separate cultivars which would in turn increase the risk of aphids landing within the crops (Ragsdale *et al.* 2001).

Understanding of vector lifecycle

A clear understanding of the lifecycle of vector aphid species is important in virus control. *M. persicae* in the Columbia Basin, Northwest USA overwinters in the egg stage on peach and almond trees (Pike and Thomas 2002). Aphids hatch in spring, multiply through several generations and then migrate to herbaceous summer hosts including potato. However, more recently it was discovered that nymphs and adults also overwinter on weeds such as common mallow, flixweed, tumble mustard, shepherd's purse and storksbill (Pike and Thomas 2002). Aphids generally colonise the undersides of leaves, and in mild winters are reproductively active, e.g. multiplying by more than sevenfold on mallow, storksbill and shepherd's purse over the 1999-2000 winter season (Pike and Thomas 2002). However, a widespread survey indicated that these weeds rarely harboured virus and aphids migrating to potato acquired virus from infected crops or volunteer potato rather than the weeds on which they overwintered.

2.7.4 Control of PVX and PVS in seed potato in British Columbia

Wright *et al.* (1976) outlined virus control strategies in seed stocks in British Columbia during the 1970's aimed mainly at controlling PVS and PVX. PLRV had

been adequately controlled in basic seed stocks by using seed control areas – isolated, interior valleys surrounded by mountains and beyond the flight range of aphids from where ware and processing potato crops were grown and hence isolated from major sources of virus inoculum. Tuber indexing and unit planting, winter testing, roguing, aphid control, top killing and family selection had all contributed to the control of PLRV. However despite these practices, PVS and PVX remained ubiquitous in North American cultivars. Heat therapy and meristem tip culture were used to produce the first virus-free clones of several cultivars in 1967, which became known as the Vancouver collection. Other accessions were received, mainly from around the USA and Canada (Wright 1987). During the 1970's many provinces in Canada and several States in the USA adopted a program to rid major varieties of PVS and PVX. This process was facilitated by a change in the Canadian seed potato certification regulations in 1969, which established a 'flush-through' system, which precluded the planting of field-grown tubers for the production of pre-Elite class of seed (Wright 1987). Elite classes were created which preceded the Foundation and Certified classes and Elite seed growers in Pemberton and Cariboo areas of British Columbia planted virus free stem cuttings each year. The production system started with the use of heat therapy and meristem tip culture to produce virus free clones. The process was described by Wright (1987). Briefly, 2-3 tip cuttings (6-8 cm long) were taken, lower leaves removed and each cutting planted in sterilised soil. After 4-6 weeks, the potted cuttings were placed under fluorescent lights (16 hr daylength) and day/night air temperatures of 36°C and 33°C respectively. Stem tips were removed after 2 weeks to promote axillary shoots. At 6-10 weeks after heat therapy, shoot tips about 15 cm long were cut from plants and axillary buds observed under a dissecting microscope. Rudimentary leaves were removed until the two youngest leaf primordia remained.

The meristematic tip (0.3-0.6 mm long) was then cut off and transferred to liquid medium. Each shoot could provide up to 12 meristem tips. Most buds developed roots and shoots within two months of culture and rooted plantlets could be moved to soil when more than 3 cm long (Wright 1987). Care was taken to avoid contact between accessions that might potentially allow transmission of PVS and PVX. Tests for PVS and PVX were conducted within a few days after each plantlet was transplanted to soil and one plantlet of each accession was then grown on for further propagation. Stem cuttings were then taken from each virus-free clone. Six to ten cuttings multiplied over three years produce approximately 2000 lb (907.2 kg) of tubers, sufficient to produce an acre of Elite 3. The Vancouver Research Station estimated requirements of growers three years into the future and planted sufficient stem cuttings to meet expected demand. Since 1974, most stem cuttings have been produced on seed growers' farms (Wright 1987). Tissue-cultured transplants are grown on in greenhouses to produce mini-tubers for field planting (Wright 1987). During the summer, each plant was tested for PVS and PVX. At harvest the tubers (pre-Elite) were stored in family groups (Wright *et al.* 1976). A sample of tubers was grown on in the greenhouse during the winter and tested for PVX, PVS, PSTVd, PVY and PLRV. However, after 19 years of testing none of the latter three pathogens had been found and tests after 1986 were continued only for PVS and PVX (Wright 1987). In the spring, tubers were planted in an isolated block separated from other families by a distance of 1.8 metres (Wright *et al* 1976). Knives and rubber gloves used in planting were sterilised with quaternary ammonia before each tuber was cut. Wright (1987) reported that at that (later) time nine tubers from each accession were planted and separated by a distance of 3 metres, suggesting that tubers were no longer cut into seed pieces. During the second summer, each plant was again tested for PVS and

PVX and if detected, the entire family was discarded. Tubers from non-infected families were then bulked and classed Elite 1. The following year they were planted in tuber units and progeny harvested (Elite 2) and the process repeated for another year to produce Elite 3 that were sold for the production of Foundation class. Samples of 250 leaflets/10 acres (up to a maximum of 1000 leaflets) were collected in a predetermined pattern from Elite 2, Elite 3 and Foundation classes during the growing season and tested for PVS and PVX by a serological test (Wright *et al.* 1976). A leaflet from every hill was tested for plots eligible for pre-Elite and Elite 1 classification (Wright 1987). For fields eligible for other classes, groups of 25 leaflets are collected from plants along diagonals of each field, with 10, 20, 30 or 40 groups collected from fields of 4 ha or less, 4-8 ha, 8-12 ha or more than 12 ha respectively (Wright 1987). Since 1985 the ELISA test has been used (Wright 1987). Leaflets were stacked in lots of 25 and two sets of discs were removed with a 15 mm cork borer. One set was stored while the other was ground and tested for virus as a composite sample. If a positive sample was found, each of the discs of the stored set were tested individually to estimate the virus incidence. An estimate of virus incidence was obtained using statistical tables to give confidence intervals. For example if 5/250 leaflets tested positive (2.0%) then there was a 95% chance that between 0.4% and 3.6% of the field plants were infected (Wright *et al.* 1976). In 1969 only 1.4% of the acreage of Elite 3 and Foundation classes were virus tested in Pemberton and Cariboo, British Columbia. However by 1976, some 85.1% of the acreage was tested. In 1976, 4994 pre-Elite stem cuttings, 549 Elite 1 families, 27 acres of Elite 2, 373 acres of Elite 3 and 109 acres of Foundation were tested for PVS and PVX. Virus was not detected in pre-Elite or Elite 1 classes and was not detected in 91% of Elite 2, 88% of Elite 3 or 36% of Foundation acreage. The highest

incidence was 11% of plants in one sample (6.6-16.7% in field) suggesting that the scheme was successful at reducing PVS and PVX (Wright *et. al.* 1976).

The effectiveness of the control program was assessed by examining the virus status of the Elite 3 seed that is marketed from Elite seed farms (Wright 1987). PVX or PVS was found in 12 of the 22 Elite seed farms in Pemberton and Cariboo between 1974 and 1986. Control of PVX and PVS was usually achieved by the flush through production system. In 1986, PVX was at the minimum level (0.0-1.5%) in all 22 Elite farms and all but one farm had the minimum level of PVS (Wright 1987). By tracing back the source of infection, Wright (1987) was able to show that PVS on one Elite seed farm had been transmitted from Elite 3 to Elite 1 or Elite 2 plantings in 1975 and from Elite 3 to Elite 2 fields in 1977 and 1978. The source of virus was probably due to a failure to detect very low incidence of virus in leaf samples or that the spread of infection occurred too late in the season to be detected by samples taken mid-season (Wright 1987).

Surveys were conducted for PVS and PVX between 1982 and 1985 in 435 fields of Foundations seed, Certified seed and table stock planted with, or derived from Elite 3 seed (Wright 1987). In most cases, most lots that had the minimum level of PVS and PVX at the Elite 3 class produced crops with no increase in virus content (Table 2.13). Furthermore after two further years of bulking up, some 85% of seed lots that had the minimum virus level at the Elite 3 class had no further increase in virus. In cases where higher levels of reinfection occurred, the source of virus was probably from outside of the seed lot. Since new infections were detected during the mid-summer testing it was assumed that infection had occurred during the seed cutting operation

(Wright 1987), which is known to occur for PVS (Franc and Banttari 1984) and for PVX (Mai 1947). Spread of PVS on a contaminated seed cutting machine was demonstrated in two Norgold Russet seed lots (Table 2.14). This highlighted that the flush-through seed production system can control PVS and PVX, but only if due care and sanitation are undertaken to prevent transmission at planting.

This scheme proved effective at preventing PVX contamination of early generation seed potato, but Wright (1987) noted that it had not been as successful at controlling PVS, except in one province.

Table 2.13. Virus status of Elite 3 seed potato in Pemberton and Cariboo, British Columbia and after growth for one or two years in other areas (adapted from Wright 1987).

	Year	No. fields	PVS % (confidence interval)
SPCA Elite 3 (18 cvs.) tested 0.0-1.5% PVS	PVS status of 288 fields one year after planting:	271	0.0-1.5% PVS
		12	0.0-5.1% PVS
		5	1.1-7.2% PVS
	PVS status of 118 fields two years after planting:	100	0.0-1.5% PVS
		13	0.0-5.1% PVS
		5	0.1-9.7% PVS
SPCA Elite 3 (18 cvs.) tested 0.0-3.5% for PVX	PVX status of 312 fields one year after planting:	296	0.0-1.5% PVX
		13	0.0-3.5% PVX
		3	1.4-9.7% PVX
	PVX status of 132 fields two years after planting:	119	0.0-1.5% PVX
		7	0.0-4.6% PVX
		6	1.4-9.7% PVX

Table 2.14. Transmission of PVS during mechanical cutting of tubers and virus incidence in subsequent crop (Wright 1987).

PVS in seed lot prior to tuber cutting	Seed cutter	No. of fields (cv. Norgold Russet)	PVS in growing crops expressed as a confidence interval
0.0-1.5%	Clean	5	0.0-1.5%
0.0-1.5%	PVS contaminated[1]	1	1.9-7.2%
0.0-1.5%	PVS contaminated[1]	1	±15%

[1] Contaminated by prior use on the same day to cut seed tubers from a field with approximately 20% incidence of PVS.

2.7.5 Control of PVX and PVS in seed potato in Western Australia

Historically, the seed production system in Western Australia had remained practically unchanged for more than 60 years (Wilson and Jones 1990). Originally production of seed potato in Western Australia involved generally one cultivar (cv. Delaware) summer planted in coastal swamplands of wind-exposed areas. Crop rotation was not utilised in the initial scheme, and elimination of unharvested tubers was assumed to occur through sheep foraging (grazing) and natural winter flooding. Management of potential virus spread involved: i) the planting of large selected tubers, ii) visual inspections during the growing season, iii) roguing of symptomatic plants, and iv) the application of aphicide to minimise aphid populations. Wilson and Jones (1990) found PVS and PVX to be prevalent and incidence within crops was high (up to 100% in some cases) in old seed stocks in Western Australia. This

highlighted the inadequacies of this seed production scheme to control these mechanically transmitted viruses (Wilson and Jones 1990).

The Western Australian scheme has been changed considerably and now involves a 'flush through', limited generation scheme based on the National Standard (Dr. R.A.C. Jones, DAFWA *personal communication*). The key components are the release of virus free minitubers and the growing of seed crops in isolation from ware crops. The Western Australian scheme also involves a three row gap to ensure no plant contact between generations. Two inspections are conducted during the growing season at flowering and pre-senescence. For G2 (sown) crops, 500 leaves are collected per generation per site at the second inspection and bulk tested in lots of 10 leaves. Samples are tested for PVS, PVX, PLRV and TSWV by ELISA and for PLRV by an immunoblotting technique. Tuber testing is also conducted with an eye from the rose end of tubers excised and grown on in the greenhouse prior to testing. The virus tolerances at first and second inspection for G1 (sown) are 0.10 and 0.01 respectively, for G2 (sown) are 0.25 and 0.10 respectively and for G3 (sown) are 1.0 and 1.0 respectively. In addition later generation crops are also tested if to be exported to Sri Lanka and Mauritius.

The success of the new scheme in controlling viruses can be seen by comparing current virus levels with those recorded historically. A survey of potato tubers harvested from seed crops grown in the Albany swamp region of Western Australia was conducted during 1987/1988 (Wilson and Jones 1990). PVX was detected in 22/23 crops, with 13/23 crops having incidences above 90%. PVS was detected in 20/23 crops, with 9/23 having 80% incidence or greater and PLRV was detected in

4/23 crops at incidences of 2% or less (Wilson and Jones 1990). During the monitoring of G2 (sown) crops conducted during 2003, the viruses PVS, PVX, PLRV, TSWV and PVY occurred in only 0.04%, 0.04%, 0.07%, 0.6% and 0% of 10,450 samples respectively (Dr. R.A.C. Jones, DAFWA, *personal communication*).

3. Prevalence and incidence of PVS and PVX in seed potato in Tasmania

3.1 Introduction

Maintenance of pathogens at low incidence in seed potato is necessary to ensure sustained crop yields and quality and to maintain the competitiveness both nationally and internationally of the potato industry in Tasmania. Production of seed potato in Tasmania involves planting minitubers and growing seed crops over four subsequent seasons or 'generations' (G1 to G4) to produce sufficient seed tubers for ware production. The seed certification scheme was introduced into Tasmania in the 1930s (Taylor 2003). Each generation is visually inspected by DPIW seed certification officers during the season and certified if disease incidence is below set thresholds of the National Standard (Chapter 2, Section 2.6.2). However certain viral pathogens such as PVS and PVX produce none, or only mild symptoms under field conditions and cannot be reliably detected by visual inspection. These pathogens are readily detected by serological testing, but this is currently not conducted as part of potato seed certification in Tasmania.

Until recently virus incidence in Tasmanian seed potato has been assumed to be very low, with the exception of sporadic and isolated reports of PLRV and TSWV. This has been attributed to isolation afforded by being an Island State and to the Tasmanian seed potato certification scheme. However, a limited survey conducted by DPIW, Simplot Australia Pty. Ltd., McCain Foods (Australia) Pty. Ltd. and Harvest Moon

Forth Farm Produce Ltd., to assess the presence of viruses in Tasmanian seed potato crops during the 2001/2002 season detected PVS, PVX and PLRV in 27%, 7% and 7% of crops respectively (Kirkwood 2003a). This survey was based on low sample numbers collected from a limited number of fields, and therefore indicated only the presence or absence of virus. In addition, the low sample numbers probably identified virus infections only in those fields with a high incidence.

The findings of the limited survey conducted during the 2001/2002 season in Tasmania seed potato stock prompted a comprehensive survey to be undertaken the following season (2002/2003 season). An additional survey was conducted in 2003/2004 growing season to assess virus incidence of PVS and PVX in early generation seed potato crops (G2). The main objectives of this study were to provide: i) a comprehensive survey of virus status in Tasmanian seed potato crops during 2002/2003 to provide an assessment of the prevalence and incidence of PVS and PVX; ii) to monitor virus incidence and prevalence of PVS and PVX in early generation Tasmanian seed potato crops (G2) crops in 2003/2004 season; iii) assess potential differences between generations of the same crop in the same year; iv) determine if differences of virus incidence occur between different potato growing regions of Tasmania. Information gained from these surveys will provided valuable information for the Tasmanian industry and assist in developing management strategies for reducing viruses status with the aim of eliminating virus from seed potato stocks in Tasmania.

3.2 Materials and methods

3.2.1 Sampling

During the 2002 potato growing season (November 2002 – April 2003) over 90% of seed potato fields (G1-G4) in Tasmania were surveyed for prevalence and incidence of PVS and PVX. During the 2003 potato growing season (November 2003 – April 2004) all G2 seed potato fields were surveyed for prevalence and incidence of PVS and PVX.

2002/2003 survey

In the 2002 season, 225 seed potato fields were assessed for PVS and 232 fields for PVX. Fifty fields were sampled by the author and certification officers from DPIW sampled the remaining fields. From each field, a single leaflet was collected (between 50-300) from different plants at flowering (mid January- early April) from arbitrarily chosen locations along transects at field edges and along irrigator and spray rig runs within the crop. A traditional 'W' sampling pattern was considered impractical due to the size of plants at flowering time, steep slope of some fields and the height of potato mounds. In addition, the sampling strategy was chosen to minimise movement through a crop that may contribute to mechanical transmission of these viruses. Field hygiene practices were adopted to reduce the risk of virus transmission between fields, which included the use of disposable boot covers between fields and washing of all equipment and hands with disinfected after sampling each field. Cultivars tested including Russet Burbank, Shepody, Ranger Russet, Kennebec, Pink Eye and various

fresh market cultivars. Leaflet samples were stored at 5°C for no more than 3 days before virus testing.

2003/2004 survey

During the 2003 potato growing season, 107 G2 seed potato fields were sampled using the same sampling procedure and hygiene practices outline above for the 2002 season. Leaflet samples (between 50-300) were collected at flowering (20 January 2003 - 7 April 2004) by certification officers (DPIW). Cultivars sampled include Russet Burbank, Ranger Russet, Pink Eye, Bintje, Kennebec, Victoria, Bismark, Granola, Carrera and Shepody. Leaflet samples were stored at 5°C for no more than 3 days before virus testing.

3.2.2 Virus testing

Virus incidence was estimated from grouped samples using the Gibbs and Gower technique (1960) (Section 3.2.4). This method maximised the number of samples collected from the field and minimised the number of samples tested. The 300 leaflet sample collected from each field was split into batches of 10 leaflets, which were arranged on top of each other with the smaller ones centred. The top half of the leaflets was cut off at right angles to leaflet veins and discarded. A further two cuts were made on each side of the vein at 45° angle to form a blunt arrowhead shape. A strip (2-3 mm wide and 2-3 cm long) was cut from the cut surface running at right angles to the veins. This method ensured that all 10 leaflets comprised part of the sample for extraction and virus testing. Scissors were sterilised in 10% household bleach solution and wiped clean between each group of leaves. Each sample of 10

leaflets was crushed between two rollers in a motorised leaf press and virus tested for PVS and PVX by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) using commercial antisera (Agdia Inc. Elkart, IN, USA) (Appendix II). Plant material samples for this chapter and all other chapters were considered positive if absorbance was greater than the mean absorbance of the negative controls plus three times the standard deviation of the buffer only (Sutula *et al.* 1986).

Virus testing of samples collected from 50 fields and 27 fields during 2002 and 2003 seasons, respectively, were assessed at the Cuthbertson Research Laboratories located at the Tasmanian Institute of Agricultural Research, University of Tasmania, Cradle Coast Campus, Burnie, Tasmania. The remainder of the samples were processed by staff at DPIW, New Town Research Laboratories, Hobart, Tasmania.

3.2.3 Validation of ELISA sensitivity

Validation of the serological technique, DAS-ELISA (Appendix II) was conducted to detect one PVS/PVX infected leaflet in a batch containing an additional nine non-infected (healthy) leaflets. Virus free cv. Russet Burbank plants were grown from certified minitubers sourced from DPIW (Stoney Rise, Tasmania). Plants were grown under greenhouse conditions in 40 mm pots containing commercial potting mix. Plants were maintained in aphid proof cages for a growth period of 8 weeks. PVS and PVX isolates were obtained from Simplot Growers' Line trial 2005. Six different isolates of PVS and PVX were used to assess the sensitivity of ELISA. Two PVS isolates were sourced from Wynyard and Scottsdale, two PVX isolates and two co-infected (PVS and PVX) isolates were sourced from Riana. A total of 90 healthy

leaflet samples were virus tested and bulked into 10 groups. One infected leaflet was added to each group of nine healthy leaflets.

3.2.4 Estimation of virus incidence

In most cases, 300 leaflets were sampled from each field. Where samples numbers obtained from fields were greater than 100, samples were bulked tested in batches of 10 and virus tested by DAS-ELISA (Appendix II). When less than 100 samples were obtained from fields, samples were tested individually. The formula of Gibbs and Gower (1960) was used to provide an estimate of the true incidence of infection from each crop. In this technique:

N is the number of grouped samples (30)

i is the number of leaflets in each grouped sample (10)

R is the number of grouped samples that give a positive virus test

p is the proportion of infected plants in the crop being studied

p^* is the maximum likelihood estimate of p

$p = 1\text{-}p$ is the proportion of uninfected plants in the population and $q^* = 1\text{-}p^*$ is the estimate of q.

The probability that none of i leaflets is infected is q^i and the probability of a positive virus test when i leaflets is tested is $1\text{-}q^i$. The estimate q^* is given by:

$$R/N = 1\text{-}q^{*i} \quad \text{Therefore } p^* = 1\text{-}q^* = 1\text{-}(1\text{-}R/N)^{1/i}$$

Equation 3.1

At low to moderate virus incidence the Gibbs and Gower formula gives a good estimate of the actual virus incidence in the sample. However at high incidence the formula does not give an accurate estimate (Figure 3.1). Due to limitations of the Gibbs and Gower formula at high virus incidence, a subsample of 20 leaflets was re-tested individually by ELISA in crops with high incidence of PVS in bulked samples. The incidence obtained from testing the individual leaflets was compared to that estimated by the Gibbs and Gower on bulked samples.

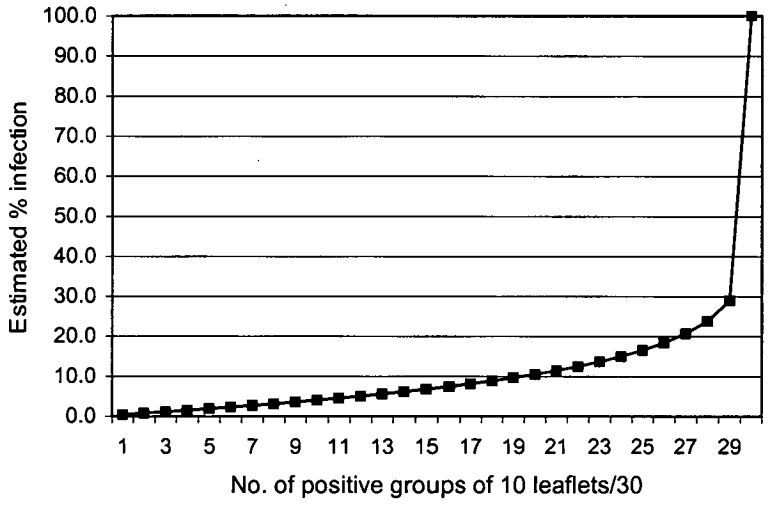


Figure 3.1. Relationship between the number of positive groups of leaflets (30 groups of 10 leaflets) and the virus incidence as estimated by the Gibbs and Gower formula.

3.2.5 Analysis of survey results

To compare virus incidence across locations, the Tasmania seed production areas were divided into seven different geographic regions (based on seed certification regions set by DPIW, Figure 3.2) and the incidence of virus between them compared statistically. The estimated incidence of PVS and PVX were compared using generalised linear modelling (Genstat Version 6).

Figure 3.2. Approximate location of seed production regions used to examine the effect of geographic location on virus incidence.

[1] Calder, East Yolla, Henrietta, Highclere, Lileah, Natone, Nietta, Preston, Riana, Ridgley, Smithton, Somerset, South Riana, Stowport, Tewksbury, Upper Natone, Waratah, West Pine, Wynyard, Yolla.

[2] Flowery Gully, Frankford, Lower Barrington, Parkham, Sheffield, Wilmot.

[3] Branxholm, Bridport, Lebrina, Ringarooma, Scottsdale, St. Helens, Tullendeena, West Scottsdale, Winnaleah.

[4] Blessington, Campbell Town, Cressy.

[5] Cranbrook, Little Swanport, Swansea.

[6] Broadmarsh, Buckland, Colebrook, Dysart, Kempton, Mt. Seymour, Nala, Runnymede, Sorrel, Stonor, Tunnack, Whiteford.

[7] Ellendale, New Norfolk, Ouse, Ranelagh, Woodstock.

3.2.6 Virus incidence in different generations of seed potato

To determine any change in virus incidence with time, virus incidence was assessed among different generations of seed potato crops. Field site selection criteria was based upon virus present on farms in the previous season and included selection of crops from a range of different locations. PVS and PVX incidence was tested from leaflet samples collected from 5 seed potato fields at flowering (early January 2003 - late April 2003) from arbitrarily chosen locations along irrigator and spray rig runs and field edges within the crop. Sampling and hygiene practices were previously outlined in Section 3.2.1. In field 1 (Yolla), 300 leaflets were collected from different plants from each generation (G) of G1, G2 and G3. Field 2, 3 and 4 were located at Scottsdale, Bridport and Burnie, respectively. One hundred leaflets were collected from all G1 crops in each of Field 2, 3 and 4. In addition, 300 leaflets were collected from each G2, G3 and G4 crop. Field 1, 2 and 4 were of the seed potato cv. Russet Burbank, while field 3 was Ranger Russet. In field 5 (Burnie) 100 and 300 leaflets samples were collected from G2 and G3, respectively of seed potato cv. Shepody. Leaflets samples were stored at 5°C and leaflets were individually tested for PVS and PVX incidence by DAS-ELISA (Appendix II).

3.3. Results

3.3.1 2002 Survey - Prevalence and incidence of PVS and PVX

Potato virus S

PVS was prevalent in Tasmanian seed potato and occurred at high incidences in some crops (Table 3.1). PVS was detected in 150/225 (66.7%) of crops tested, with 131 of these crops (58.2%) above the National Standard for Certification of seed potato of 1% (Table 3.1). The mean estimated incidence (% of infected plants) of the 225 crops surveyed was 17.9%. Some crops had very high incidences, with 28 crops (12.4%) having incidences of PVS above an estimated 50% (Table 3.1). However, 189 crops (84.0%) had low to moderate virus incidence or no detectable PVS incidence (<25%) (Table 3.1). PVS was not detected in 75/225 (33.3%) crops tested (Table 3.1).

PVS was detected in cvs. Russet Burbank, Ranger Russet, Shepody, Kennebec and Pink Eye (Table 3.2). PVS was also detected in less common cv. including Bintje, Nadine, and other crops of mixed cultivars (Table 3.3). PVS was not detected in cvs. Atlantic, Bismark, Granola and Nooksack (Table 3.3).

Potato virus X

PVX was less prevalent than PVS and occurred at lower incidence (Table 3.1) in seed potato in Tasmania. PVX was detected in 30/232 (12.9%) of crops surveyed, with 19/231 (8.2%) of crops having incidences above the National Standard (Table 3.1). The estimated mean incidence of PVX of the 231 crops surveyed was 0.3%. Most

infected crops had low incidence, with only 2 crops having above 10% infection (Table 3.1). The highest recorded incidence in any crop was 18.2%.

PVX was detected in cvs. Russet Burbank, Shepody and one field of mixed cultivars. PVX was not detected in cvs. Ranger Russet , Kennebec, Pink Eye, Atlantic, Bintje, Granola, Nadine or Nooksack (Tables 3.2, 3.3). PVX was detected in 27/137 (19.7%) of crops of the cv. Russet Burbank (Table 3.2), with 18/137 (13.1%) crops above the National Standard and highest incidence of crops tested was 6.7%. (Table 3.2). In Shepody crops tested, PVX was detected in 2/22 (9.1%) crops with one crop above National Standard of 1% incidence. The highest incidence of crops tested was 2.6% (Table 3.2).

Table 3.1. Frequency of occurrence of crops in different categories of *Potato virus S* (PVS) and *Potato virus X* (PVX) virus incidence as estimated by Gibbs and Gower (1960) formula from batch testing groups of leaflets from Tasmanian seed potato crops collected during 2002/2003 growing season.

	Virus	
	PVS	**PVX**
Number of crops with:		
No detectable virus	75	201
0-1%	19	11
1-10%	53	17
11-25%	42	2
26-50%	8	0
≥51%	28	0
Total crops surveyed	225	232
Mean estimated incidence (%)[1]	17.9	0.3
Standard Deviation (%)	32.0	1.6

[1]Caution is required with mean estimated incidence. As previously mentioned, cases where the Gibbs and Gower formula gave an estimate of 100% infection were in the range of 40-100% when individual leaflets were retested.

Table 3.2. Virus incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) in different cultivars of Tasmanian seed potato collected during the 2002/2003 growing season.

	# crops	mean virus incidence (%)	Range of virus incidence within crops (%)	# crops with incidence:					
				=0	<1%	≥1%	≥10%	≥25%	≥50%
Potato virus S									
Russet Burbank	130	18.2	0-100	26	6	98	58	25	21
Shepody	22	9.6	0-100	13	1	8	2	2	2
Ranger Russet	19	12.7	0-100	10	1	8	7	4	1
Kennebec	10	22.5	0-100	5	0	4	2	2	2
Pink Eye	5	4.8	0-23.7	3	1	1	1	0	0
Mixed	24	6.8	0-100	10	6	7	5	1	1
Various fresh	15	11.0	0-100	8	3	5	3	3	1
Total crops	225								
Potato virus X									
Russet Burbank	137	0.5	0-18.3	110	12	18	2	0	0
Shepody	22	0.2	0-2.6	20	1	1	0	0	0
Ranger Russet	19	0	0	19	0	0	0	0	0
Kennebec	10	0	0	10	0	0	0	0	0
Pink Eye	5	0	0	5	0	0	0	0	0
Mixed	24	0.02	0-0.7	23	1	0	0	0	0
Various fresh	15	0	0	15	0	0	0	0	0
Total crops	232								

Table 3.3 Presence of *Potato virus S* (PVS) and *Potato virus X* (PVX) in Tasmanian fresh market seed potato cultivars collected during the 2002/2003 growing season.

Cultivar	No. crops tested	PVS	PVX
Atlantic	1	-[1]	-
Bintje	2	+[2]	-
Bismark	1	-	-
Granola	1	-	-
Nadine	2	+	-
Nooksack	1	-	-
Other[3]	31	+	+

[1] - = virus not detected by DAS-ELISA

[2] + = virus detected by DAS-ELISA

[3]Other includes other cultivars and mixtures of cultivars (e.g. Nicola and Kennebec).

3.3.2 Test of the Gibbs and Gower procedure at high virus incidence

As previously mentioned, the Gibbs and Gower formula becomes inaccurate as an estimate of virus incidence at high incidence. To test this, in 18 cases where 30/30 grouped samples were positive, 20 leaves were re-selected at random from the original sample and retested individually. The mean percentage PVS infection for the 18 crops was 78.6% (standard deviation = 19.2%) with a range of 40-100%. Retests the individual leaves in 6/18 cases (level of infection in retest leaves 100%), 9/18 cases (infection ≥80%), 16/18 cases (≥50%) and 2/18 cases (infection 40%≥50%≤) show in the majority of cases infection of retested leaves were greater than 50%. Therefore the highest category used in Table 3.1 was 50%.

3.3.3 2002 Survey – Difference in virus incidence between geographic regions

When all cultivars surveyed were considered together across all 7 regions, the incidence of PVS in region 3 was statistically higher than all other regions (Table 3.4). Regions 1, 2, 4 and 6 had intermediate incidence of PVS, with regions 5 and 7 having low incidence. PVX was found at low incidence and only in regions 1 and 2.

Table 3.4. Incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) across all cultivars of seed potato crops in different geographic regions of Tasmania (2002/2003 season).

		Mean incidence (%) of:	
Region	No. crops	PVX	PVS
1	105	0.68	12.2 bc[1]
2	29	0.12	14.3 b
3	33	0.0	57.8 a
4	13	0.0	18.3 bcd
5	7	0.0	0.10 e
6	29	0.0	8.2 bcde
7	16	0.0	1.7 de
P	-	not significant	<0.001

[1] Means within columns followed by the same letter are not significantly different.

To examine differences in incidence in Russet Burbank across regions there were sufficient crops for analysis in regions 1 to 3 only. The mean incidence of PVS in region 3 (60.2%) was significantly higher than that in regions 1 and 2 (Table 3.5). There was significantly greater mean incidence of PVX in region 1 compared with region 3, with the incidence in region 2 being intermediate between these (Table 3.5).

Table 3.5. Incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) in Russet Burbank seed potato crops in different geographic regions of Tasmania (2002/2003 season).

		Mean incidence (%) of:	
Region	No. crops	PVX	PVS
1	82	0.83 a[1]	13.9 a
2	19	0.15 ab	19.2 a
3	23	0.0 b	60.2 b
P	-	<0.001	<0.001

[1] Means within columns followed by the same letter are not significantly different.

3.3.4 2003 Survey – Prevalence and incidence of PVS and PVX in G2 crops

Potato virus S

PVS was detected in 45/107 (42.1%) of the crops tested, with 39 crops (36.5%) above the National Standard for Certification of Seed Potato of 1% incidence (Table 3.6). However, the majority of crops had low to moderate incidence with 101/107 crops (94.4%) having either no detectable PVS or less than 25% incidence (Table 3.6).

PVS was detected in cvs. Russet Burbank, Ranger Russet, Pink Eye, Bismark and Nicola with several crops having high estimated PVS incidence (Tables 3.7, 3.8). PVS was not detected in Kennebec, Shepody, Bintje or other crops tested (Tables 3.7, 3.8).

Potato virus X

PVX was detected in 5/107 (4.7%) of crops tested (Table 3.6), with 4 crops (3.7%) above the National Standard for Certification of Seed Potato of 1% incidence. One

crop had an estimated incidence over 50% (Table 3.6). The majority of crops tested had no detectable PVX (95.3%). PVX was detected in cvs. Russet Burbank and Pink Eye. PVX was not detected in other cultivars (Table 3.7), although only a small number of crops of different cultivars were sampled (Table 3.8).

Table 3.6. Frequency of occurrence of crops in different categories of *Potato virus S* (PVS) and *Potato virus X* (PVX) incidence as estimated by Gibbs and Gower (1960) formula from batch testing groups of leaflets collected from generation 2 Tasmanian seed potato crops collected during 2003/2004 growing season.

	Virus	
	PVS	**PVX**
Number of crops with:		
No detectable virus	62	102
0-1%	6	1
1-10%	19	3
11-25%	14	0
26-50%	1	0
≥51%	5	1
Total crops surveyed	107	107
Mean estimated incidence (%)[1]	8.1	1.0
Standard Deviation (%)	21.5	9.7

[1]Caution is required with mean estimated incidence. As previously mentioned, cases where the Gibbs and Gower formula gave an estimate of 100% infection were in the range of 40-100% when individual leaflets were retested.

Table 3.7. Virus incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) in common cultivars of Tasmanian seed potato collected during 2003/2004 growing season.

	Potato cultivar:				
	Bintje	Pink Eye	Russet Burbank	Ranger Russet	Other[1]
PVS					
Total crops tested	4	5	37	10	42
Number of crops with:					
No detectable virus	4	3	11	5	31
0-1%	0	0	3	1	2
1-10%	0	1	11	0	6
11-25%	0	0	10	1	3
26-50%	0	0	1	1	0
≥51%	0	1	2	2	0
Mean estimated incidence (%)[2]	0	20.2	12.9	23.5	1.4
Std. Deviation (%)	0	44.6	23.8	43.5	3.5
PVX					
Total crops tested	4	5	37	10	42
No. crops with:					
No detectable virus	4	4	33	10	42
0-1%	0	0	1	0	0
1-10%	0	0	3	0	0
11-50%	0	0	0	0	0
≥51%	0	1	0	0	0
Mean estimated incidence (%)[2]	0	20.0	0.2	0	0
Std. Deviation (%)	0	44.7	0.6	0	0

[1]Other includes other cultivars and mixtures of cultivars.

[2]Caution is required with mean estimated incidence. As previously mentioned, cases where the Gibbs and Gower formula gave an estimate of 100% infection were in the range of 40-100% when individual leaflets were retested.

Table 3.8. Presence of *Potato virus S* (PVS) and *Potato virus X* (PVX) in less common seed potato cultivars grown in Tasmania during the 2003/2004 growing season.

Cultivar	No. crops tested	PVS	PVX
Bismark	1	+[2]	-[1]
Granola	1	-	-
King Edward	1	-	-
Nicola	1	+	-
Red Rascal	2	-	-

[1] - = virus not detected by DAS-ELISA

[2] + = virus detected by DAS-ELISA

3.3.5 Generational study

PVS was detected in all generations and cultivars of Field 2, 3, and 5. PVS was not detected in G1 of Field 1 (Table 3.9), however, G2 had 2.2% incidence of PVS and there was an increase of 18.4% between G2 and G3. PVS incidence was higher in G2- than G1 (9.3%) and higher in G3 than G2 (11%) in Field 2. However, PVS incidence was 10.2% lower in G4 than G3 (Table 3.8). In Field 3 PVS incidence was 5.1% higher in G2 than G1 and 1.0% higher in G3 than in G2. PVS incidence of both G3 and G4 was 9.6%. In Field 4 PVS incidence was 16.1% lower in G2 than in G1, with a G3 1.5% less than G2, and a further 12.9% PVS incidence lower in G4 than in G3. In Field 5 PVS incidence was 9.5% higher in G3 than in G2.

PVX was detected in G2 of Field 1, G2, G3 and G4 of Field 4 and G2 and G3 of Field 5 (Table 3.9). PVX was detected in G2 but not in G4 of Field 1. PVX was not detected

in Field 2 or 3. In Field 4 PVX incidence was equally 20.6% higher in G3 and G4 than in G2. In Field 5 PVX was 5.3% higher in G3 than in G2. PVX was detected in cvs. Russet Burbank and Shepody, but not in Ranger Russet.

Table 3.9. Mean incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) in different generations of five Tasmanian seed potato fields during 2002/2003 growing season.

Field, generation (G) and cultivar	Mean PVS incidence (%)	Mean PVX incidence (%)
Field 1- Russet Burbank		
G1[1]	0	0
G2[1]	2.2	18.3
G3[1]	20.6	0
Field 2 - Russet Burbank		
G1[2]	0.3	0
G2[1]	9.6	0
G3[1]	20.6	0
G4[1]	10.4	0
Field 3 – Ranger Russet		
G1[2]	3.5	0
G2[1]	8.6	0
G3[1]	9.6	0
G4[1]	9.6	0
Field 4 - Russet Burbank		
G1[2]	0.3	0
G2[1]	16.4	3.1
G3[1]	14.9	23.7
G4[1]	2.2	23.7
Field 5 - Shepody		
G2[2]	1.8	3.5
G3[1]	11.3	8.8

[1]300 leaflets and [2]100 leaflets virus tested by DAS-ELISA

3.4 Discussion

PVS was prevalent in Tasmanian seed potato crops, occurring in 66.7% of a range of generations and cultivars in 2002 and 42% of a range of G2 cultivars in 2003. PVS occurred at relatively high prevalence and incidence, with 58.2% and 36.5% of crops above the National Standard for Certification of Seed Potato of 1% incidence in 2002 and 2003 seasons, respectively. A higher mean incidence of PVS was detected in crops in the North East region of the State (region 3) in the 2002 season. The reason for this is not apparent, but perhaps reflects earlier introduction of PVS into crops in this region, differences between regions in seed handling practices or potential aphid vector activity.

PVX was moderately prevalent in Tasmanian seed potato crops, occurring in 12.9% of crops tested, with 8.2% crops above the National Standard of 1% incidence for the 2002 season. PVX was less prevalent in G2 crops tested during the 2003 season, occurring in 4.7% crops. However virus incidence in early generation crops is of concern because of the possibility of virus increase in later generations. In the 2002 season PVX occurred in a limited range of cultivars and at low incidence, with only two crops having an incidence above 10%. In the 2003 season one G2 crop had greater than 50% incidence of PVX. In addition, PVX occurred in only a few of the seed production areas for both seasons.

In a three generational study conducted during 2003, this study did not monitor single seed lots over a number of generations, alternatively different generations grown on a farm were sampled in a single season. However, there was a general trend for increase

in virus in succeeding generations. PVS was detected at low incidence in three early generation crops (G1) at 0.3-3.5%. The finding of virus in such early generation material is of major concern given the potential for virus transmission and increase over generations of a total of five fields (G1-G4). In most cases PVS incidence increased in each subsequent generation (G1 to G3) for each field. In fields 2, 3 and 4 a levelling off or decrease of PVS incidence was detected between G3 and G4. PVX was detected in G2, G3 and G4 of three, two and one field(s), respectively. Two fields had no detectable PVX in any generation. Although the same seed lot was not followed from one season to the next, alternatively all generations were assessed from virus in the same season and the increase may be due to an inability to detect virus in the previous (earlier) generation.

In the late 1960's a virus free Russet Burbank line was imported into Tasmania from Canada (Taylor 2003) and the suspected increase in virus incidence in Tasmanian seed potato crops in recent years may have also arisen from the gradual relaxation in standards required for seed potato. Initially early generations of seed were grown in isolation at the DPIW Tewkesbury Potato Station located in central Tasmania at an altitude above 220 metres and thus providing protection against aphids and associated potato diseases. However, the development of a minituber facility at DPIW Stoney Rise, Devonport and a move by seed growers to produce all generations of their planting stock, led to the sale of the Tewkesbury Potato Station in 2000 (Taylor 2003). Initially, early generation seed was grown in isolation at above 220 metres altitude to avoid potential aphid vector activity, mainly for the control of PLRV. However, in 1996 the height requirement was reduced to 180 metres. In 1997, there was a further relaxation, which allowed seed crops to be grown below 180 metres, however,

mandatory testing for PLRV by ELISA was required for certification of seed. Mandatory testing involved a sample of 300 leaflets collected from lots above 5000 plants, and a sliding scale for lots of less than 5000 plants. Leaflets were bulked in 30 lots of 10 and tested by ELISA. As PLRV was detected only infrequently, the requirement for virus testing was later abolished.

It is notable that despite a relaxation in the altitude requirement, PLRV, which is exclusively aphid borne in a persistent manner (by *Myzus persicae, Macrosiphum euphorbiae* and *Aulacorthum solani*), continues to occur only sporadically and at very low incidence in Tasmanian seed potato (Hay *et al.* 2005). *M. persicae* can also transmit some strains of PVS. Therefore the apparent increase in PVS, which can be mechanically transmissible and aphid transmissible, might not be a factor of reducing the requirement for altitude for early generation seed so much as reducing the requirement for isolation. That is, growing early generation seed crops near to potentially infected later generation or ware crops on the same farm increases the potential risk of mechanical transmission (eg. on machinery) or of non-persistent aphid transmission. A further factor that may have contributed to the increase in mechanically transmitted viruses is the use of contract harvesters and the move towards four centralised seed-cutting facilities in Tasmania over the past 10-20 years. A large number of seed crops are processed at these facilities, which therefore provide a potential means of spreading mechanically transmitted viruses such as PVS and PVX between crops and an increase within crops as they are cut. Other potential reasons for virus increase in Tasmania compared to previous decades (Taylor 2003) include the use of non-certified virus-infected fresh market crops such as cultivars Pink Eye and Dutch Cream by home gardeners and small fresh market producers. This

non-certified virus-infected seed may act as a source of inoculum for seed crops, especially as the latter can now be grown in lower altitude areas that are becoming increasingly urbanised.

The higher incidence in seed potato compared to virus in previous decades (Talyor 2003) may be also be explained in part by late season infection associated with a lack of mature plant resistance to PVS, seed cutting or spread early the next season. Franc and Banttari (1996) suggested the rapid reinfection of PVS in potato with a PVS isolate from Minnesota was due in part to the absence of mature plant resistance. Detection of PVS infection in potato foliage by ELISA required a 13-20 days time period after the initial mechanical inoculation of PVS onto a leaf, although PVS moved out of the inoculated leaf within 24 hours of inoculation (Franc and Banttari 1996). Although other studies have reported an increase in virus incidence in later generations compared to earlier generations, the generation study conducted in Tasmania did not follow a seedlot over a number of seasons.

At present incidence of viruses other than PVS in Tasmania is low (Hay *et al.* 2004). PLRV, PVY and PVX are therefore likely to be more easily eradicated than PVS in the short term. It is critical that these potato viruses be contained in Tasmania to prevent co-infections occurring. Overseas studies indicate that PVS alone has only a minor impact on yield (up to 15% loss). However, PVS-infected crops found in combination with viruses such as PVX, have reported yield losses of up to 40% (Stevenson *et al.* 2001).

In other states of Australia virus surveys for PVS and PVX have been limited. In South Australia virus testing between 1999-2005 by Horticultural Pathology Diagnostic Service (South Australia) has been reported but did not include certified seed potato crops. Although only 38 leaf samples and 25 tuber samples randomly collected were assessed for PVS the detection rate (26.3% and 72%, respectively) was higher than for other viruses tested such as PLRV, TSWV, PVX and PVY. Also within a crop the highest incidence occurred for PVS and PVX (Hall and Wicks 2005).

During 1979-1980 a survey was conducted for PVS and PVX of fresh market certified seed in Victoria and PVS was detected in two of nine Foundation crops at an estimated incidence of 0.105% and 100%, respectively (Moran *et al.* 1983). PVX was not detected. PVS was detected in seven of 20 certified seed crops with an estimated incidence of infected crops ranging from 0.105% to 100%. PVX was detected in five of 20 certified seed crops with an estimated incidence with infected crops ranging from 0.105 % to 1.597%. During 1980-1981, PVS and PVX were detected in one of seven Foundation crops with an estimated incidence of 0.211% (Moran *et al.* 1983). PVS was detected in four of 21 certified seed crops with an estimated incidence ranging from 0.325% to 100%, while PVX was detected in eight of 21 certified seed crops with an estimated incidence ranging from 0.103% to 1.189% (Moran *et al.* 1983). Results of a recent survey conducted in seed crops in Victoria during 2005/2006 growing season found PVS to be widespread across all seed potato growing districts. PVS was detected in 219/1547 (14.2%) of samples tested, however, PVX was not detected in any crops tested (Blackmore 2006).

A survey of commercial potato crops in the Lockyer Valley, Queensland detected PVS and PVX in 57% and 15% of crops, respectively, with PVY occurring sporadically (Holmes and Teakle 1980). In a later survey of Queensland potato, PVS was detected in tuber samples taken from one of three crops of certified seed potato from NSW, but not in six crops from Victoria, while PVX was not detected (Jafarpour *et al.* 1988). In tests of tubers from commercial crops, PVS was present in seven and PVX in eight out of 11 crops, with an average incidence of 3.3% and 3.5%, respectively.

Recent information of PVS and PVX virus status obtained from comprehensive surveys in other states of Australia is limited with the exception of Victoria (previously mentioned) and Western Australia. In the past PVS and PVX were prevalent and at high incidence (some crops up to 100%) in the seed potato grown under the seed scheme in Western Australia. The old scheme consisted predominantly of one cultivar (cv. Delaware) with summer planting in coastal swamplands wind-exposed areas. Under this scheme crop rotation of was not utilised and elimination of unharvested potatoes relied on sheep foraging and natural winter flooding. Management strategies to control virus spread included: i) rouging of symptomatic plants; ii) planting of large selected tubers; iii) visual inspections during the growing season; and iv) the application of aphicide to minimise aphid populations (Wilson and Jones 1990). These management strategies were not adequate to minimise virus in seed crops in Western Australia under the old seed certification scheme, particularly latent viruses such PVS, as infected plants were not identified during visual inspections of the crop during the growing season.

In Western Australia a rigorous seed certification scheme which includes mandatory serological testing for common viruses including PVS and PVX has been implemented and considerable changes to the seed scheme have occurred resulting in a reduction in virus prevalence and incidence in seed potato in Western Australia. The reduction in virus in seed stocks as been attributed to the introduction of a "flush through', limited generation scheme based on the National Standard (Dr. R.A.C. Jones, DAFWA, *personal communication*). Key components of this scheme include isolation of seed crops from ware crops and the release of virus free minitubers. The Western Australian scheme also involves: i) two seed inspections conducted during the growing season, at flowering and pre-senescence; ii) three-row gap between plantings of different generations to ensure no plant contact occurs; iii) all G2 (sown) crops serologically tested for virus, with 500 leaves collected from each generation and site, and iv) leaf samples tested for PVS, PVX, TSWV by ELISA and for PLRV by a petiole immunoblotting technique (samples bulk tested in lots of 10 leaves). In addition tubers testing is conducted with an eye from the rose end of tubers excised and grown on in the greenhouse prior to testing. Virus tolerances at first and second inspection for G1 (sown) are 0.10 and 0.001, respectively, for G2 (sown) are 0.25 and 0.10, respectively, and for G3 (sown) are 1.0 and 1.0, respectively. Later generation crops are also tested if they are to be exported to Sri Lanka and Mauritius

As mentioned the new scheme has been successful at reducing the prevalence and incidence of viruses. A survey of potato tubers harvested from seed crops grown in the Albany swamp region of Western Australia was conducted during 1987/1988 (Wilson and Jones 1990). PVX was detected in 22/23 crops, with 12/23 crops having incidence above 90%. PVS was detected in 4/23 crops at incidence of 2% or less (Wilson and

Jones 1990). In 2003 during the monitoring of G2 (sown) crops of the 10 450 samples virus tested PVS, PVX, PLRV, TSWV and PVY occurred in only 0.04%, 0.04%, 0.07%, 0.6% and 0% of the samples, respectively (Dr. R.A.C. Jones, DAFWA, *personal communication*).

4. Strain characterisation of PVS isolates from Tasmanian seed potato

4.1 Introduction

4.1.1 Strains of PVS

Globally, two major strains of PVS have been characterised, the ordinary (PVS^O) and the Andean strain (PVS^A). Identifying which major strains of PVS are present in Tasmania will be important in developing management strategies for reducing virus incidence in seed potato stocks. Until the 1980s only one PVS strain was considered to infect potato cultivars (Wetter 1971). Novel strains of PVS were reported in the early to mid 1980s in Holland (Rose 1983), U.S.A. (Slack 1981; Jones 1983). U.K. (Slack 1981), Germany (Slack 1983; Dolby and Jones 1987) and later in New Zealand (Fletcher 1996). PVS^O and PVS^A strains can be distinguished by symptom expression on inoculated *Chenopodium quinoa*. As previously discussed (Section 2.2.2), subsequent symptoms following mechanical inoculation of PVS^O to *C. qunioa* produce chlorotic lesions restricted to inoculated leaves, that generally appear approximately 4-8 days after inoculation. In addition to local lesions on inoculated leaves, PVS^A produces systemic symptoms of mottle or necrosis on the upper leaves (non-inoculated leaves) at approximately 12-21 days after inoculation. PVS^A is considered more efficiently transmitted by aphids or contact than PVS^O. Systemic infection of other indicator species such as the domestic

tomato (*Lycopersicon esculentum* Mill.) has also been used to identify the presence or absence of PVS^A and to distinguish between other potato viruses.

4.1.2 Tomato (*Lycopersicon esculentum*) and kangaroo apple (*Solanum laciniatum*) as host species of PVS

Previous studies have shown isolates of PVS from North America and Europe were unable to infect *L. esculentum* (Slack 1983). On this basis, mixed infections of PVS and PVM have been separated as PVM readily infect *L. esculentum* (Wetter 1972; Bangall 1956 cited in Slack 1983). Kowalska and Was (1976) were also unable to inoculate a wild high altitude tomato cultivar, *L. chilense* with PVS isolates. In contrast others have demonstrated susceptibility of *L. esculentum* to PVS with many PVS isolates (Slack 1983). Symptomless systemic infections of PVS^A but immunity to PVS^O infection in *L. esculentum* have been reported (Brunt and Loebenstein 2001; Slack 1983). It is not known whether PVS isolates from Tasmanian seed potato can infect *L. esculentum.*

Solanum laciniatum Ait. is a native perennial species of Tasmania and a potential host of PVS. *Solanum laciniatum* is a soft wooded shrub and is a member of the Solanaceae family, with the common name of "kangaroo apple". *S. laciniatum* colonise disturbed patches of land and is commonly found in shady damp sites of wet sclerophyll (< 1000 mm rainfall per annum) areas in Tasmania, New South Wales, Victoria, South Australia and New Zealand. Growth habit is vigorous and shrubs may grow to 3 m. Leaf shape ranges from narrow-lanceolate (10-25 cm long) to several lanceolate with coarse lobes

near the base. Purple flowers (5cm diameter) appear on long stalks on the plant during the spring and summer months and flowering tends to last for several months (Figure 4.1). Berries are approximately 3 cm in length and of an ellipsoid shape and when ripe are bright orange-yellow (Cameron 1996; Curtis 1993; Jatisantienr 1983).

a) **b)**

Figure 4.1. *Solanum laciniatum* seedling at 16 days after emergence (a) and Mature *S. laciniatum* with lanceolate leaves and purple inflorescence (b).

In New Zealand, Russian Federation, Egypt, India and some other countries *S. laciniatum* has been cultivated since the mid 1960s. Solasodine (Lancaster and Mann 1975 cited in Thomas 1976), a steroid alkaloid contained in the young foliage of *S. laciniatum* is extracted and used commercially for the manufacture of drugs such as contraceptives (Thomas 1976; Donaldson 1983).

In New Zealand viruses including PVX, PVA and PVY, *Cucumber mosaic virus* (Family *Bromoviridae*, genus *Cucumovirus*, CMV), *Tobacco mosaic virus* (Family not assigned,

genus *Tobamovirus*, TMV) and TSWV have been shown to infect *S. laciniatum* (Thomas 1976). Symptoms are more severe on leaves of *S. laciniatum* plants co-infected with PVX and PVY than infections with individual virus (Thomas 1979). Thomas (1979) suggests virus infection of potato diseases such as PVX and PVY may be high in *S. laciniatum* where virus infected potato crops are grown adjacent. In Tasmania *S. laciniatum* has not been assessed as a potential weed host for PVS, nor for PVX isolates obtained from Tasmanian seed potato.

The aims of this study were to determine the major strains of PVS (PVS^{O}/PVS^{A}) present in seed potato grown in Tasmania by assessing, a) symptom expression on indicator species, and b) serological testing of PVS inoculated to the indicator species, *C. quinoa*, and c) RT-PCR and restriction enzyme analysis of capsid gene sequences. In addition, studies were conducted to determine if *L. esculentum* or *S. laciniatum* are susceptible hosts of PVS isolates obtained from Tasmanian potatoes.

4.2. Materials and methods

4.2.1 PVS isolates

A total of 84 PVS isolates were collected from seed potato foliage grown from tubers collected from different potato seed lines of a Simplot Australia Pty. Ltd. Growers' Line Trials during the 2003 and 2005 seasons. PVS isolates were investigated to determine presence of any strain variants present in Tasmania. PVS isolates were maintained as potato plants grown from PVS-infected tubers. Plants were maintained at 20°C in aphid-proof cages, and plant foliage was separated by a plastic bag placed around each plant. Foliage was tested for PVS prior to inoculation studies by DAS-ELISA (Appendix II).

Fifty eight PVS isolates were used for inoculation to the indicator species, *C. quinoa* (Table 4.1). Twenty-one PVS isolates were further assessed by RT-PCR and restriction fragment length polymorphism (RFLP) analysis of capsid gene. In addition 23 and 15 PVS isolates were used for inoculation studies on *L. esculentum* and *S. laciniatum*, respectively (Table 4.1).

Tubers of cv. Russet Burbank were collected from individual seed potato lines from the Growers' Line trials on 10 April 2003 and 12 May 2005. Tubers were maintained at 4°C for five months. Tuber dormancy was broken by placing tubers in individual containers at room temperature (10-15°C) for 4 days prior to planting of tubers. Tubers were planted in 200 mm pots in commercial potting mix and maintained at ~20°C under greenhouse

conditions. One leaflet from each plant was collected and serologically tested for PVS and PVX by DAS-ELISA (Appendix II) prior to inoculation experiments. PVS isolate code, isolate origin, regional zone and year of collection are shown in Table 4.1 (see Figure 3.9, p. 103 for zone reference). Nine PVS isolates were co-infected with PVX (Table 4.1).

Table 4.1. Isolate code, origin, regional zone and year of collection of *Potato virus S* (PVS) isolates and *Potato virus X* (PVX) co-infected with PVS isolates obtained from individual seed potato lines from Growers' Line trials conducted by Simplot (Australia) Pty. Ltd for characterisation by serological, indicator species and RT-PCR-RFLP.

PVS isolate code	Indicator experiments			RT-PCR and RFLP analysis of capsid gene	Region/Town (zone)[3]	Year of isolate collection
	Chenopodium quinoa	*Lycopersicon esculentum*	*Solanum laciniatum*			
TAS05-NW2.18	-[1]	-	+[2]	-	Wilmot (2)	2005
TAS05-NW3.9	-	-	+	-	Sheffield (2)	2003
TAS03-NW4.3	+	-	-	+	Sheffield (2)	2003
TAS05-NW4.5	+	+	-	-	Sheffield (2)	2005
TAS05-NW5.15	-	-	+	-	Yolla (1)	2005
TAS03-NW6.4	+	-	-	+	South Riana (1)	2003
TAS05-NW6.13	-	-	+	-	South Riana (1)	2005
TAS05-NW7.4	+	+	-	-	Preston (1)	2005
TAS05-NW7.5	+	+	-	-	Preston (1)	2005
TAS03-NW7.5	+	-	-	-	Upper Natone (1)	2003
TAS03-NW10.3	+	-	-	-	Wilmot (2)	2003
TAS05-NW11.2	+	+	-	-	Sheffield (2)	2005
TAS03-NW13.2	+	-	-	-	Wilmot (2)	2003
TAS05-NW13.5	+	+	-	-	Riana (1)	2005
TAS05-NW13.9	-	-	+	-	Riana (1)	2005
*TAS03-NW15.1	+	-	-	-	Riana (1)	2003
*TAS05-NW16.1	+	+	-	-	Riana (1)	2003
TAS03-NW16.5	+	-	-	-	Riana (1)	2005
TAS03-NW17.2	+	-	-	-	South Riana (1)	2003

Table 4.1. cont.

PVS isolate code	Indicator experiments			RT-PCR and RFLP analysis of capsid gene	Region/Town (zone)[3]	Year of isolate collection
	Chenopodium quinoa	*Lycopersicon esculentum*	*Solanum laciniatum*			
TAS05- NW17.2	+	+	-	-	Ridgley (1)	2005
TAS03-NW17.4	+	-	-	-	South Riana (1)	2003
*TAS03-NW19.5	+	-	-	-	Riana (1)	2003
TAS05-NW21.2	-	-	+	-	Sheffield (2)	2005
TAS03-NW22.1	+	-	-	-	Sheffield (2)	2003
TAS05-NE22.4	+	+	-	-	Scottsdale (3)	2005
TAS03-NW23.1	+	-	-	+	Tunnack (6)	2003
TAS04-NW24.1	-	+	-	-	Riana (1)	2004
TAS05-NW24.3	+	+	-	-	Sheffield (2)	2005
TAS03-NW24.4	+	-	-	+	Riana (1)	2003
TAS05-NE25.1	+	+	-	-	West Scottsdale (3)	2005
TAS03-NW25.4	+	-	-	+	Riana (1)	2003
TAS03-NW28.1	+	-	-	-	Wilmot (2)	2003
TAS05-NW28.1	-	+	+	+	Riana (1)	2005
TAS05-NW28.3	+	+	-	-	Upper Natone (1)	2005
TAS05-NW29.13	-	-	+	-	Natone (1)	2005
TAS03-NW31.5	+	-	-	-	Calder (1)	2003
TAS05-NW31.5	+	+	-	-	Riana (1)	2005
TAS05-NW31.10	-	-	+	-	Riana (1)	2005
TAS05-NW32.17	-	-	+	-	South Riana (1)	2005
*TAS03-NW33.3	+	-	-	-	Riana (1)	2003

Table 4.1. cont.

PVS isolate code	Indicator experiments			RT-PCR and RFLP analysis of capsid gene	Region/Town (zone)[3]	Year of isolate collection
	Chenopodium quinoa	*Lycopersicon esculentum*	*Solanum laciniatum*			
TAS05-NE34.5	-	-	+	-	Scottsdale (3)	2005
TAS03-NW35.2	+	-	-	+	Preston (1)	2003
TAS03-NW35.3	+	-	-	-	Preston (1)	2003
*TAS03-NW37.5	+	-	-	+	Natone (1)	2003
TAS05-NW37.9	-	-	+	-	Irishtown (1)	2005
*TAS05-NW37.19	-	-	+	-	Irishtown (1)	2005
TAS03-NW40.4	+	-	-	-	Lower Barrington (2)	2003
TAS03-NW40.5	+	-	-	+	Lower Barrington (2)	2003
TAS05-NW41.2	+	+	-	-	Yolla (1)	2005
TAS03-NW41.3	+	-	-	+	Yolla (1)	2003
*TAS03-NW42.4	+	-	-	-	Riana (1)	2003
TAS05-S43.7	-	-	+	-	Woodstock (7)	2005
TAS05-S47.4	-	-	+	-	Woodstock (7)	2005
TAS05-S48.1	+	+	-	-	Woodstock (7)	2005
*TAS03-NW48.4	+	-	-	-	Riana (1)	2003
TAS05-S48.5	-	-	-	+	Woodstock (7)	2005
*TAS05-NW49.1	+	+	-	-	Riana (1)	2005
TAS03-NE49.2	+	-	-	-	Scottsdale (3)	2003
*TAS03-NE49.5	+	-	-	-	Scottsdale (3)	2003
*TAS03-NE50.1	+	-	-	+	Branxholm (3)	2003
TAS05-NW50.2	+	+	-	+	Riana (1)	2005

Table 4.1. cont.

PVS isolate code	Indicator experiments			RT-PCR and RFLP analysis of capsid gene	Region/Town (zone)[3]	Year of isolate collection
	Chenopodium quinoa	*Lycopersicon esculentum*	*Solanum laciniatum*			
TAS03-NE50.3	+	-	-	+	Branxholm (3)	2003
TAS03-NE52.1	+	-	-	-	Scottsdale(3)	2003
TAS03-NE53.2	+	-	-	+	Scottsdale (3)	2003
TAS03-NE56.1	+	-	-	-	Riana (1)	2003
TAS05-NE56.1	+	-	-	-	Scottsdale (3)	2005
TAS03-NE56.3	+	-	-	-	Riana (1)	2003
TAS04-NE56.3	-	+	-	-	Scottsdale (3)	2004
TAS05-NE57.4	+	+	-	-	Tullendeena (3)	2005
TAS05-NE57.5	+	+	-	-	Tullendeena (3)	2005
TAS05-NE58.2	+	+	-	-	Winnaleah (3)	2005
TAS03-NE58.5	+	-	-	-	Winnaleah (3)	2003
TAS03-NE59.2	+	-	-	-	Winnaleah (3)	2003
TAS03-NE59.3	+	-	-	+	Winnaleah (3)	2003
TAS03-NE60.1	+	-	-	-	St. Helens (3)	2003
TAS05-NE60.1	+	+	-	-	St. Helens (3)	2005
TAS04-NE-MC4	-	-	-	+	Scottsdale (3)	2004
TAS04-NE-MC5	-	-	-	+	Scottsdale (3)	2004
TAS04- NE-MC6	-	-	-	+	Scottsdale (3)	2004
TAS04- NE-MC12	-	-	-	+	Scottsdale (3)	2004
TAS04- NE-PE18	-	-	-	+	Preston (1)	2004

[1] - = PVS isolate not tested

[2] + = PVS isolates tested

[3] Seed growing geographical location in Tasmania shown in Figure 3.2 (Chapter 3, p. 103)

* = PVS isolate co-infected with PVX

4.2.2 Inoculation protocol

Prior to inoculation, seedlings of *C. quinoa, L. esculentum* and *S. laciniatum* were maintained in darkness for 24 hours to optimise susceptibility of assay plants as described by Hiruki (1975). Leaf material from PVS-infected source plants (1g) were macerated in 10 ml phosphate buffer with an autoclaved pestle and mortar. Phosphate buffer (0.1M phosphate buffer, pH 7.6) contained 13 ml of 0.2 M solution (31.2g $NaH_2PO_4.2H_2O$, made up to 1 L with distilled water) and 87.0 ml of 0.2 M solution (28.39g Na_2HPO_4, made up to 1 L with distilled water), diluted to a total of 200 ml with distilled water to give pH 7.6 (Cruickshank 1965). Each PVS inocula was rubbed liberally onto three leaves of five replicate plants of *C. quinoa* or *L. esculentum* or *S. laciniatum* seedlings that had been previously dusted with the abrasive celite. New disposable gloves were used for each PVS isolate. Inoculated leaves were identified by tying red wool loosely around the petiole. Inoculated leaves were rinsed with distilled water immediately after inoculation to remove excess celite. Plants were placed in the dark for 24 hours then maintained at ~20°C in the greenhouse for 30-55 days. Control plants were inoculated with phosphate buffer and celite only.

4.2.3 PVS indicator species - *Chenopodium quinoa*

C. quinoa seeds were planted in commercial seed raising mix and maintained under greenhouse conditions at 20-25°C for approximately three weeks or until seedlings were at the 8-10 leaf stage. Three leaves of each of five *C. quinoa* seedlings were inoculated

(Section 4.2.2) with PVS isolates listed in Table 4.1. *C. quinoa* plants were visually scored at four-day intervals for four weeks for lesion development on inoculated leaves and symptoms on non-inoculated leaves.

4.2.4 Other potential host species of PVS

Lycopersicon esculentum

Commercial seeds of *L. esculentum* cv. *Grosse lisse* were planted in commercial seed raising mix and maintained at ~20°C in the greenhouse for 3 weeks or until seedlings were at the 5-7 leaf stage. Two isolates (Table 4.1) were inoculated (Section 4.2.2) onto three leaves of each of five *L. esculentum* seedlings on 5 September 2004 and a further 21 isolates inoculated on 7 October 2005. Plants were observed at 4-day intervals for four weeks for symptom expression. Controls consisted of inoculation of phosphate buffer only onto five *L. esculentum* seedlings. Leaf samples of each of the three inoculated leaves and three upper (non-inoculated) leaves were collected on 5 October 2004 and 7 November 2005.

Solanum laciniatum

S. laciniatum seeds were obtained from Wildseeds Tasmania (Sorell, Tasmania). Seeds were planted on 7 December 2005 in commercial seed raising seed mix and maintained at 25°C for 4 weeks or until seedlings were 20 cm high and at the 3-4 leaf stage. Fifteen Tasmanian PVS isolates were inoculated (Section 4.2.2) on 9 January 2006 and 11 January 2006 onto three leaves of each of five *S. laciniatum* seedlings. *S. laciniatum*

plants were grown in a glasshouse at ~20°C and symptom expression on inoculated leaves and upper (non-inoculated) leaves was observed every fourth day over 55 days.

4.2.5 Assessment of seed transmission of PVS in *Solanum laciniatum*

Twenty seeds were collected from PVS-inoculated and control (phosphate buffer only) of *S. laciniatum* plants on 3 July 2006, seeds were stored in labelled bags at room temperature (~15°C). Twenty seeds from each PVS-inoculated *S. laciniatum* plant collected in the previous season were planted in seed trays in commercial potting mix on 2 December 2006. Two leaves from twenty *S. laciniatum* plants were collected on 5 March 2007, 94 days after planting and virus tested for PVS by DAS-ELISA (Appendix II) within 48 hours of collection.

4.2.6 Serological identification of viral isolates

For each of the five inoculated *C. quinoa* and *L. esculentum* plants and five control plants (phosphate buffer only), three inoculated leaves and three upper (non-inoculated) leaves were individually virus tested for PVS and PVX by DAS-ELISA (Appendix II). *C. quinoa* leaves were collected at 24 days after inoculation (DAI) and *L. esculentum* leaves were collected 30 DAI. Leaves collected for virus testing were stored at 4°C and processed within 48 hours of collection date.

One inoculated leaf from each of the five inoculated *S. laciniatum* and five control plants for each of the 15 PVS isolates tested was individually tested by DAS-ELISA (Appendix II) on 14 February 2006 (37 DAI). Three upper (non-inoculated) leaves of each of the five inoculated *S. laciniatum* and five control plants for each of the 15 PVS isolates tested were individually tested by DAS-ELISA (Appendix II) at 53-55 days after inoculation on 2 March 2006.

4.2.7 RT-PCR-RFLP comparison of PVS strain capsid gene

A restriction fragment length polymorphism (RFLP) technique was adopted to address some of the ambiguity between symptom expression and serological results of *C. quinoa* inoculated with PVS isolates. This method of differentiating between major strains of PVS was developed by Dr. Jason Scott and Dr. Frank Hay as part of a University of Tasmania Institute Research Grants Scheme project and has been utilised for determining strain characterisation of PVS in Tasmanian seed potato. The technique was based on a reverse transcriptase polymerase chain reaction (RT-PCR) technique similar to that developed by Heldák (2001).

RT-PCR-RFLP

RNA sequences of the coat protein gene of PVS were obtained from GenBank including one PVS^{O}, PVS^{A} and 12 unidentified PVS strains and aligned with Clustal W. PVS sequences were compared to sequences from a known PVS^{O} and PVS^{A} strain using programs NEIGHBOR and DNADIST from the PHYLIP package of programs. Distance

analysis was conducted using the neighbour-joining method of NEIGHBOR, based on Kimura 2-parameter distance matrices generated with DNADIST. All 12 untyped sequences of PVS on GenBank grouped with the published sequence of PVS^{O}. Published sequence of PVS^{A} grouped in a second clade.

PCR primers for the amplification of both PVS strains were designed, targeting conserved regions of the coat protein gene.

(Sense primer)	5' ATGCCGCCTAAACCAGATCC 3'
	3' TACGGCGGGTTTGGCCTAGG 5'
(Anti- sense primer)	5' TGATTGCGCACAATCTCAGC 3'
	3' ACTAACGCGTGTTAGAGTCG 5'

The sense primer comprises part of the primer S2 used by Heldák (2001). The predicted size of the amplicon from these probes was 863 bp. The specificity of these to the PVS coat protein gene was tested by a BLAST search of the GenBank database for compatible sequences. No sequence other than those of the PVS coat protein were detected to match both probes sufficiently to allow amplification.

To differentiate between PVS strains, restriction enzyme maps of all sequences were generated, using the software BIOEDIT. Two enzymes (*Sac*I and *Sac*II) were predicted to cut amplified DNA of the two PVS^{A} strains in one place, but not that of PVS^{O}, resulting in digestion products of 162 and 701 bp, and 363 and 500 bp, respectively. Four enzymes

(*Ahd*I, *Eco*NI, *Psp5*II and *San*DI) were predicted to cut amplicons from the PVS^{O} strains once, but not the PVS^{A} strains. *Ahd*I was predicted to give products of 383 and 480 bp, the enzyme *Eco*NI was predicted to give products of 385 and 478 bp. The enzymes *Sac*I, *Sac*II, and *Ahd*I were selected to use in these studies to differentiate PVS strains due to price and availability.

RNA extraction

PVS isolates were obtained from plant foliage grown from infected tubers (Table 4.1). RNA extraction was conducted using the QIAGEN Plant Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's protocol. Briefly total RNA was extracted by grinding 50 mg of PVS-infected leaf tissue in liquid Nitrogen to a powder in a mortar and pestle. The powder was transferred into a 2 ml microcentrifuge tube and 450 µl of Buffer RLT (pre-mixed with beta-mercaptoethanol as directed by instructions of manufacturer) added and vigorously vortexed. Lysate was pipetted into QIA shredder column and placed in a new 2 ml microcentrifuge tube and centrifuged for 3 minutes at 13, 000 rpm. The elutant was carefully transferred to a new microcentrifuge tube. A 0.5 volume (usually 225 µl) of 96-100% ethanol was added to the solution to clear lysate, mixing by repeated pipetting. A 650 µl sample and any precipitate was added to a RNeasy mini column then placed in a 2 ml microcentrifuge tube and centrifuged for 30 seconds at 10,000 rpm. The flow through was discarded and the collection tube retained for the next step of the procedure. Added to the each RNeasy column was 700 µl Buffer RW1, followed by centrifugation for 15 seconds at 10, 000 rpm. RNeasy columns were transferred to new 2 ml collection tubes, and 500 µl Buffer RPE (diluted with ethanol as

per manufacture's instructions) added and tubes centrifuged for 15 seconds at 10, 000 rpm. Flow through was discarded, followed by 500 µl of Buffer RPE (diluted with ethanol as per manufacture's instructions) added to each column and tube centrifuged for 2 minutes at 10, 000 rpm. Flow through was discarded. RNA was eluted by transferring the RNeasy column to a new 1.5 ml microcentrifuge tube and adding 30-50 µl of RNase-free water directly onto the RNeasy silica-gel membrane. Elutant containing RNA were centrifuged for 1 minute at 10, 000 rpm and RNA was stored at -80°C.

RT-PCR was conducted using the QIAGEN One-Step RT-PCR kit according to manufacturer's protocol using conditions similar to Heldák (2001), using a Perkin Elmer Gene Amp 2400 therocycler.

i) Reverse Transcription: preheat to 50°C, 30 min. @ 50°C

ii) PCR activation: 15 min. @ 95°C

iii) PCR: 30 cycles of 1 min. denaturation @ 94°C, 1 min. annealing @ 55°C, 1 min. primer extension @ 72°C

Restriction digestion of RT-PCR product was carried out at 37°C for 3 hours. RT-PCR products (5µl) was mixed with the following:

*Sac*I: 3.8 µl water, 1 µl NE Buffer (100x), 0.1 µl BSA (100x), 0.1 µl enzyme

*Sac*II: 3.9 µl water, 1 µl NE Buffer (100x), 0.1 µl enzyme

*Ahd*I: 3.65 µl water, 1 µl NE Buffer (100x), 0.1 µl BSA (100x), 0.25 µl enzyme

Amplicons were separated in 1.5% agrose gel in 1 X TAE running buffer at 80V for 80 minutes, stained with ethidium bromide (0.5 $mg.L^{-1}$) and visualised under a UV transilluminator.

4.3. Results

Serological results by DAS-ELISA of PVS source potato plants used for inoculation experiments had absorbance values ranging between 0.743-1.945 (A_{405}nm), in comparison to healthy sap<0.08.

4.3.1. Symptom expression of PVS in *Chenopodium quinoa*

A total of 58 isolates of PVS were inoculated onto *C. quinoa* (Table 4.2). Twenty (34.5%) PVS isolates were characterised as PVS^O based on local lesions on inoculated leaves of *C. quinoa*, absence of symptoms on non-inoculated leaves, and with virus detection by DAS-ELISA in inoculated leaves only (Table 4.2). Symptoms induced on inoculated leaves included chlorotic local lesions, yellowing of leaves, yellowing tips of leaves, yellow veining and faint mottle. One of the 20 isolates of PVS was co-infected with PVX (Table 4.2), but as PVX was not detected serologically in test plants this isolate was characterised as PVS^O. A further three (5.2%) isolates of PVS were identified as PVS^A based on local lesions on inoculated leaves and mild mottling and/or chlorotic spots on non-inoculated leaves, with positive DAS-ELISA of inoculated and non-inoculated leaves (Table 4.2, Figure 4.2). Systemic symptoms induced by isolates identified as PVS^A by symptom expression on *C. quinoa* included faint mottling, several chlorotic lesions on non-inoculated leaves (Figure 4.3), very faint chlorotic lesions and yellowing on leaf tips (Figure 4.2). Systemic symptom expression on upper leaves was observed at 21 DAI. All control plants were virus free.

Fourteen (24.1%) PVS isolates were identified as PVS^{O}-like based on positive DAS-ELISA of inoculated leaves only, however, no symptoms were observed on either inoculated or non-inoculated leaves (Table 4.2). One of these isolates of PVS was co-infected with PVX, however as PVX was not detected serologically in test plants this isolate was characterised as PVS^{O}-like. Nine (15.5%) isolates of PVS were characterised as PVS^{A}-like based on positive DAS-ELISA in both inoculated and non-inoculated leaves but lack of typical symptoms. Of these, four isolates produced no symptoms on *C. quinoa* and five isolates produced symptoms on inoculated leaves only (Table 4.2). A further nine (15.9%) PVS isolates could not be characterised on the basis of symptoms, as they were co-infected with PVX and inoculated plants of *C. quinoa* were infected with both viruses. However of these, four isolates of PVS were detected serologically in upper non-inoculated leaves, while five isolates were confined to inoculated leaves only. Some differences in the ability of PVX isolates to by systemic in *C. quinoa* were also evident, with seven of the nine isolates detected serologically in inoculated leaves only. Observed symptom expression of upper leaves of plants co-infected with PVS and PVX included several chlorotic lesions on several upper leaves (Figure 4.3). Stunting of *C. quinoa* plants inoculated with PVS or PVS and PVX isolates was not observed when compared with control plants. A further three (5.2%) PVS isolates (TAS05-NW7.4, TAS05-NW10.3 and TASNW13.5) did not infect *C. quinoa* seedlings (Table 4.2), this may be due to low infection efficiency.

Table 4.2. Symptom expression of *Potato virus S* (PVS) on indicator species, *Chenopodium quinoa* used to distinguish between PVS Ordinary and Andean strains (PVS^O/PVS^A). *C. quinoa* were inoculated with PVS strains isolated obtained from seed potato in Tasmania.

Isolate code	Symptom expression		Serological result inoculated leaves				Serological result non-inoculated leaves[1]			
	Inoculated leaves	Non-inoculated leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves
PVS^O										
TAS05-NW4.5[2]	FCLL	n.s. (-)	0	0	1	4	5	0	0	0
TAS03-NW6.4[3]	YV	n.s. (-)	0	2	0	3	5	0	0	0
TAS03-NW13.2	FYL	n.s. (-)	0	0	0	5	5	0	0	0
*TAS05-NW16.1[2]	FCLL	n.s. (-)	PVS: 0 PVX: 5?	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 5 PVX: 0	PVS: 5 PVX: 5	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0
TAS03-NW16.5	FCLL	n.s. (-)	0	0	0	5	5	0	0	0
TAS03-NW17.4	CLL	n.s. (-)	0	1	3	1	5	0	0	0
TAS05-NW22.4[2]	DL	n.s. (-)	0	0	0	5	5	0	0	0
TAS03-NW23.1[3]	CLL	n.s. (-)	0	0	1	4	5	0	0	0
TAS05-NW24.3[2]	CLL	n.s. (-)	0	0	1	4	5	0	0	0
TAS03-NW24.4[3]	CLL	n.s. (-)	0	0	0	5	5	0	0	0
TAS03-NW25.4[3]	CLL	n.s. (-)	1	0	0	4	5	0	0	0
TAS03-NW35.3	CLL	n.s. (-)	1	0	2	2	5	0	0	0

Table 4.2. cont.

Isolate code	Symptom expression		Serological result inoculated leaves				Serological result non-inoculated leaves[1]			
	Inoculat-ed leaves	Non-inoculat-ed leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves
PVS^{O} cont.										
TAS03-NW40.4	CLL	n.s. (-)	0	2	1	2	5	0	0	0
TAS03-NE49.2	FCLL	n.s. (-)	0	0	0	5	5	0	0	0
TAS05-NW50.2[2,3]	FCLL	n.s. (-)	0	0	1	4	5	0	0	0
TAS03-NE56.1	CLL	n.s. (-)	0	0	1	4	5	0	0	0
TAS05-NE56.1	DL	n.s. (-)	0	0	1		5	0	0	0
TAS05-NE57.5[2]	DL/ FCL	n.s. (-)	0	0	0	5	5	0	0	0
TAS03-NE60.1	FCLL	n.s. (-)	0	0	0	5	5	0	0	0
TAS05-NE60.1[2]	FCLL	n.s. (-)	0	0	0	5	5	0	0	0
PVS^{O}-like										
TAS03-NW7.5	n.s. (+)	n.s. (-)	4	0	1	0	5	0	0	0
TAS05-NW11.2[2]	n.s. (+)	n.s. (-)	0	0	0	5	5	0	0	0
TAS05-NW17.2[2]	n.s. (+)	n.s. (-)	0	0	0	5	5	0	0	0
TAS05-NW25.1[2]	n.s. (+)	n.s. (-)	0	0	0	5	5	0	0	0
TAS05-NW28.3[2]	n.s. (+)	n.s. (-)	0	0	1	4	5	0	0	0
TAS05-NW31.5[2]	n.s. (+)	n.s. (-)	0	0	0	5	5	0	0	0
TAS03-NW35.2[3]	n.s. (+)	n.s. (-)	1	2	1	1	5	0	0	0
TAS05-NW41.2[2]	n.s. (+)	n.s. (-)	0	0	0	5	5	0	0	0

Table 4.2. cont.

Isolate code	Symptom expression		Serological result inoculated leaves				Serological result non-inoculated leaves[1]			
	Inoculat-ed leaves	Non-inoculat-ed leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves
PVS^{O}-like cont.										
TAS05-NW48.1[2]	n.s. (+)	n.s. (-)	0	0	0	5	5	0	0	0
*TAS03-NE49.1[2]	n.s. (+)	n.s. (-)	PVS: 0 PVX: 5?	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 5 PVX: 0	PVS: 5 PVX: 5	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0
TAS03-NE53.2[4]	n.s. (+)	n.s. (-)	0	1	0	4	5	0	0	0
TAS05-NE57.4[2]	n.s. (+)	n.s. (-)	0	0	0	5	5	0	0	0
TAS05-NE58.2[2]	n.s. (+)	n.s. (-)	0	0	1	4	5	0	0	0
TAS03-NE58.5	n.s. (+)	n.s. (-)	1	0	3	1	5	0	0	0
PVS^{A}										
TAS03-NW40.5[3]	CLL	n.s/fm	0	2	1	2	0	4	1	0
TAS03-NE50.3[3]	CLL/yt	vfCL	0	0	0	5	1	1	0	2
TAS03-NE59.3[3]	CLL	vfCL	0	0	0	5	3	2	0	0
PVS^{A}-like										
TAS03-NW4.3[3]	n.s. (+)	n.s. (+)	0	0	0	3	3	2	0	0
TAS03-NW17.2	n.s. (+)	n.s. (+)	0	0	2	3	3	2	0	0
TAS03-NW22.1	CLL	n.s. (+)	0	1	0	4	4	1	0	0
TAS03-NW28.1	CLL,fm	n.s. (+)	0	2	0	3	4	0	0	1
TAS03-NW31.5	n.s. (+)	n.s. (+)	0	0	2	3	3	2	0	0
TAS03-NW41.3[3]	FCLL	n.s. (+)	1	0	0	4	4	0	0	1
TAS03-NE52.1	FYL	n.s. (+)	0	0	0	5	2	0	3	0
TAS03-NE56.3	CLL	n.s. (+)	0	0	0	5	0	2	1	2
TAS03-NE59.2	n.s. (+)	n.s. (+)	2	0	1	2	3	2	0	0

Table 4.2. cont.

Isolate code	Symptom expression		Serological result inoculated leaves				Serological result non-inoculated leaves[1]			
	Inoculat-ed leaves	Non-inoculat-ed leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves
Unidentified strains										
*TAS03-NW37.5[3]	CLL	CL (PVS: - PVX:+)	PVS: 0 PVX: 0	PVS: 2 PVX: 0	PVS: 1 PVX: 0	PVS: 2 PVX: 5	PVS: 5 PVX: 2	PVS: 0 PVX: 2	PVS: 0 PVX: 1	PVS: 0 PVX: 0
*TAS03-NE50.1[3]	CLL	Yt (PVS: - PVX:+)	PVS: 1 PVX: 3	PVS: 0 PVX: 2	PVS: 0 PVX: 0	PVS: 4 PVX: 0	PVS: 5 PVX: 4	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 1
*TAS03-NW19.5	CLL	n.s. (-).	PVS: 1 PVX: 0	PVS: 1 PVX: 0	PVS: 3 PVX: 0	PVS: 0 PVX: 5	PVS: 5 PVX: 5	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0
*TAS03-NW48.4	CLL	n.s. (-)	PVS: 0 PVX: 1	PVS: 1 PVX: 1	PVS: 0 PVX: 0	PVS: 4 PVX: 3	PVS: 5 PVX: 5	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0
*TAS03-NW15.1	CLL	n.s.	PVS: 0 PVX: 1	PVS: 1 PVX: 0	PVS: 3 PVX: 0	PVS: 1 PVX: 4	PVS: 3 PVX: 5	PVS: 2 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0
*TAS03-NE49.5	fm	n.s. (+)	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 5 PVX: 5	PVS: 3 PVX: 5	PVS: 2 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0
*TAS03-NW33.3	CLL	n.s. (-)	PVS: 1 PVX: 1	PVS: 2 PVX: 0	PVS: 0 PVX: 0	PVS: 1 PVX: 4	PVS: 5 PVX: 5	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0
*TAS03-NW42.4	CLL/yl	n.s. (+)	PVS: 1 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 4 PVX: 5	PVS: 2 PVX: 5	PVS: 2 PVX: 0	PVS: 1 PVX: 0	PVS: 0 PVX: 0
*TAS03-NE49.5	fm	n.s. (+)	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0	PVS: 5 PVX: 5	PVS: 3 PVX: 5	PVS: 2 PVX: 0	PVS: 0 PVX: 0	PVS: 0 PVX: 0

Table 4.2. cont.

Isolate code	Symptom expression		Serological result inoculated leaves				Serological result non-inoculated leaves[1]			
	Inoculated leaves	Non-inoculated leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves	No. of plants with no +ve leaves	No. of plants with 1 +ve leaf	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves
Strains unable to infect plants										
TAS05-NW7.4[2]	n.s. (-)	n.s. (-)	5	0	0	0	5	0	0	0
TAS03-NW10.3	n.s. (-)	n.s. (-)	5	0	0	0	5	0	0	0
TAS05-NW13.5[2]	n.s. (-)	n.s. (-)	5	0	0	0	5	0	0	0

[1]Non-inoculated refer to upper newly expanded tip leaves

[2]PVS isolates inoculated to *Lycopersicon esculentum*

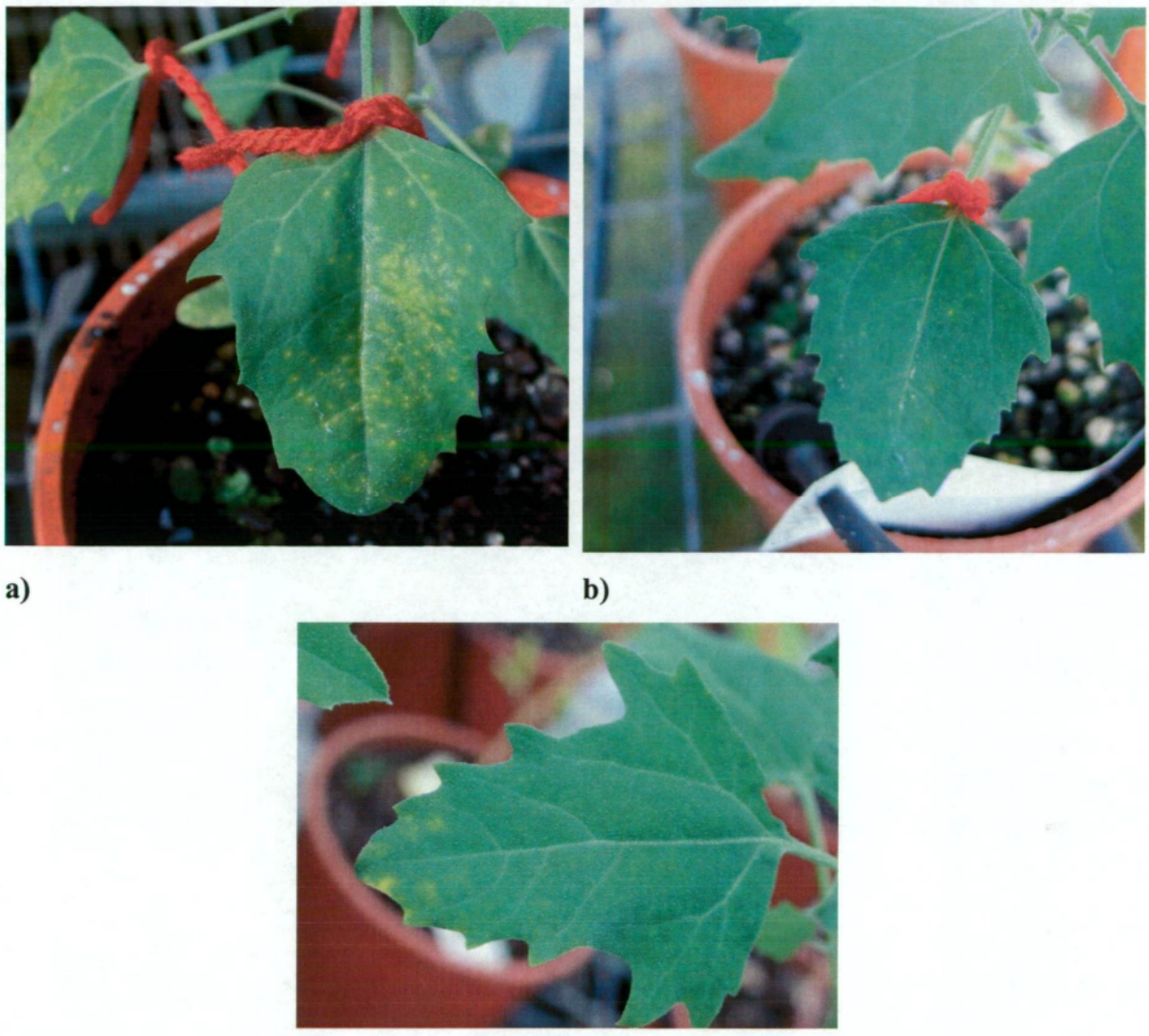
[3]PVS isolates assessed by RT-PCR and RFLP analysis of capsid gene

+ve refers to *Potato virus S*-infected leaves

Symptoms listed were confirmed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA):

n.s. = no symptom observed in leaves; + = serological infection detected; - = no serological infection detected; vf = very faint; F = faint; CLL = chlorotic local lesions in inoculated leaves; CL = chlorotic lesion; YV = yellow veining; yl = yellowing of leaves; yt = yellowing of leaf tips; m = mottling of leaves; DL = damaged leaf from inoculation process and obstructing potential symptom expression in inoculated leaves.

* PVS isolates co-infected with PVX



a) **b)**

c)

Figure 4.2. Symptom expression observed on *Chenopodium quinoa* inoculated with *Potato virus S* (PVS) isolates sourced from seed potato grown in Tasmania, **a)** chlorotic local lesions on inoculated leaf, **b)** faint chlorotic local lesions on inoculated leaf, and **c)** yellowing on leaf tip of upper (non-inoculated) leaf.

a)

b)

Figure 4.3. Symptom expression on *Chenopodium quinoa* inoculated with *Potato virus S* (PVS) isolate (TAS03-NW37.5) obtained from seed potato grown in Tasmania, a) *C. quinoa* plant showing chlorotic local lesions on inoculated leaves denoted with red wool and mild chlorotic systemic symptom on one leaf only on non-inoculated leaves 21 days after inoculation (denoted by arrow), b) upper (non-inoculated) leaf (right) showing minimal chlorotic systemic symptoms and chlorotic local lesions on inoculated leaf (right) denoted with red wool (left).

4.3.2. Other potential host species and symptom expression

Lycopersicon esculentum

None of the 23 PVS isolates inoculated to *L. esculentum* seedlings were able to infect *L. esculentum.* No symptoms were observed on inoculated or non-inoculated leaves of *L. esculentum* plants. Similarly no symptoms were observed on control plants. Serological testing for PVS verified no infection occurred in inoculated or non-inoculated leaves of *L. esculentum* or control plants. Inoculations with two isolates with co-infections of PVS and PVX resulted in symptomless PVX infection (but no PVS) within inoculated leaves only.

Solanum laciniatum

No symptom expression was observed on inoculated or upper (non-inoculated) leaves of *S. laciniatum* plants inoculated with one Tasmanian isolate co-infected with PVS and PVX isolates (Table 4.3) and no symptom expression or virus was detected on control plants. However, PVS infection was detected serologically in non-symptomatic inoculated and non-inoculated leaves for 11 and 13 PVS isolates inoculated onto *S. laciniatum* seedlings, respectively (Table 4.3).

Absorbency (A_{405} nm) values of PVS positive samples in inoculated and non-inoculated leaves ranged between 0.396 -0.792 and 0.108 – 0.967, respectively while the (A_{405} nm of healthy control plants ranged from 0.068-0.072). Two PVS isolates (TAS05-S43.7 and TAS05-S47.4) from Woodstock origin did not infect *S. laciniatum* after mechanical inoculation. PVS isolate, TAS05-NW29.13 was only serologically detected in two upper

(non-inoculated) leaves of one plant, although two inoculated leaves from two separate plants were infected (Table 4.3). All three upper (non-inoculated) leaves inoculated with PVS isolates TAS05-NW5.15 and TAS05-NW28.1 were infected with PVS (Table 4.3). For 11 PVS isolates the percentage of PVS infected leaves was higher in the upper (non-inoculated) leaves of compared to inoculated leaves (Table 4.4).

Five *S. laciniatum* seedlings were inoculated with PVS (isolate TAS05-NW37.19) co-infected with PVX. PVX was not detected in inoculated or non-inoculated leaves of *S. laciniatum* while PVS was detected in both (Table 4.3 and Table 4.4).

Table 4.3. Symptom expression of *Potato virus S* (PVS) at 53-55 days after inoculation on *Solanum laciniatum* with PVS strains isolated obtained from seed potato in Tasmania.

PVS isolate code	Symptom expression		[1]Serological result:	Serological result Non-inoculated leaves (upper leaves)			
	Inoculated leaves	Non-inoculated leaves	No. of successfully inoculated plants per isolate	No. of plants with no +ve leaves	No. of plants with 1 +ve leaves	No. of plants with 2 +ve leaves	No. of plants with 3 +ve leaves
TAS05-NW2.18	n.s. (+)	n.s. (+)	1	2	0	0	3
TAS05-NW3.9	n.s. (+)	n.s. (+)	2	1	1	1	2
TAS05-NW5.15	n.s. (+)	n.s. (+)	4	0	0	0	5
TAS05-NW6.13	n.s. (+)	n.s. (+)	4	1	0	2	2
TAS05-NW13.9	n.s. (+)	n.s. (+)	3	0	1	2	2
TAS05-NW21.2	n.s. (+)	n.s. (+)	3	1	1	0	3
TAS05-NW28.1	n.s. (+)	n.s. (+)	3	0	0	0	5
TAS05-NW29.13	n.s.(+)	n.s. (+)	2	4	0	1	0
TAS05-NW31.10	n.s. (+)	n.s. (+)	2	0	0	2	3
TAS05-NW32.1	n.s.(-)	n.s. (+)	0	3	0	1	1
TAS05-NE34.5	n.s.(-)	n.s. (+)	0	2	0	1	2
TAS05-NW37.9	n.s. (+)	n.s. (+)	1	1	1	1	2
*TAS05-NW37.19	n.s. (+)	n.s. (+)	PVS:1 PVX:0	PVS:2 PVX:5	PVS:1 PVX:0	PVS:0 PVX:0	PVS:2 PVX:0
TAS05-S43.7	n.s.(-)	n.s.(-)	0	5	0	0	0
TAS05-S47.4	n.s.(-)	n.s.(-)	0	5	0	0	0

Symptoms listed were confirmed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA):

n.s. = no symptom observed in leaves; + = serological infection detected; - = no serological infection detected.

* = PVS isolates co-infected with PVX.

+ve refers to *Potato virus S*-infected leaves

[1]One leaf of each of the five inoculated plants was serologically tested for PVS and PVX by DAS-ELISA (Appendix II).

Table 4.4. Symptom expression and percentage of *Potato virus S* (PVS) infected leaves of *Solanum laciniatum* using PVS strains isolated obtained from seed potato in Tasmania.

Isolate code	Symptom expression		Serological	
	Inoculated leaves	Non-inoculated leaves	[1]Percentage of PVS positive inoculated leaves (%)	[2]Percentage of PVS positive upper leaves (%)
TAS05-NW2.18	n.s. (+)	n.s. (+)	20	60
TAS05-NW3.9	n.s. (+)	n.s. (+)	40	60
TAS05-NW5.15	n.s. (+)	n.s. (+)	80	100
TAS05-NW6.13	n.s. (+)	n.s. (+)	80	66.7
TAS05-NW13.9	n.s. (+)	n.s. (+)	60	73.3
TAS05-NW21.2	n.s. (+)	n.s. (+)	60	66.7
TAS05-NW28.1	n.s. (+)	n.s. (+)	60	100
TAS05-NW29.13	n.s.(+)	n.s. (+)	40	13.3
TAS05-NW31.10	n.s. (+)	n.s. (+)	40	93.3
TAS05-NW32.1	n.s.(-)	n.s. (+)	0	33.3
TAS05-NE34.5	n.s.(-)	n.s. (+)	0	53.3
TAS05-NW37.9	n.s. (+)	n.s. (+)	20	60
*TAS05-NW37.19	n.s. (+)	n.s. (+)	PVS:20 PVX:0	PVS:46.6 PVX:0
TAS05-S43.7	n.s.(-)	n.s.(-)	0	0
TAS05-S47.4	n.s.(-)	n.s.(-)	0	0

Symptoms listed were confirmed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA):

n.s. = no symptom observed in leaves; + = serological infection detected; - = no serological infection detected.

* = PVS isolates co-infected with PVX.

[1]One leaf of each of the five inoculated plants was serologically tested for PVS and PVX by DAS-ELISA (Appendix II).

[2]Three upper (non-inoculation) leaflets of five inoculated plants serologically tested for PVS and PVX by DAS-ELISA (Appendix II).

4.3.3 Assessment of seed transmission of PVS in *Solanum laciniatum*

PVS was not detected in 260 *S. laciniatum* plants grown from seeds collected from PVS-inoculated *S. laciniatum* plants or controls (phosphate buffer only) from the previous season.

4.3.4 Molecular characterisation of PVS strains

The banding patterns of all 21 Tasmanian PVS isolates was as for that predicted for PVS^{O} strains i.e. the amplicon was cut by enzyme *Ahd*I into 383 and 480 bp but was not cut by *Sac*I or *Sac*II (Figure 4.4). This included five PVS isolates (TAS03-NW4.3, TAS03-NW28.1, TAS03-NW40.5, TAS03-NE52.1 and TAS03-NE59.3) which caused systemic infection and symptoms when inoculated onto *C. quinoa* (Table 4.2).

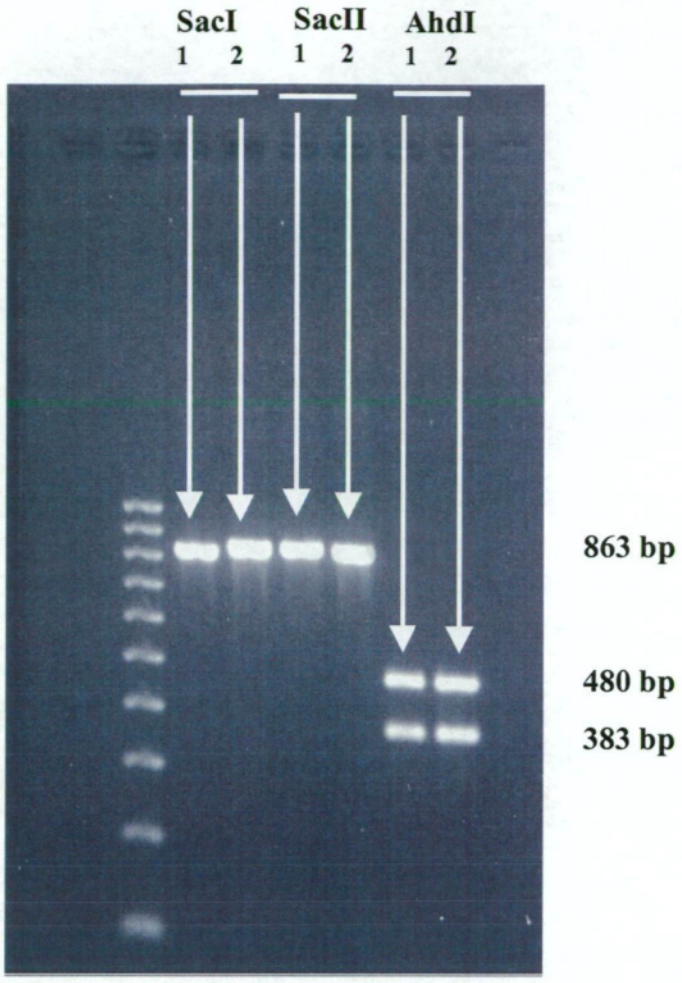


Figure 4.4. Agarose gel electrophoresis of products from RT-PCR of *Potato virus S* (PVS) isolates extracted from leaf tissue obtained from seed potato grown in Tasmania. Restriction digest of RT-PCR amplicon of two PVS isolates (TAS03-NW4.3 and TAS03-NE59.3). Lane 1 = 100 bp ladder, Lane 2 = *Sac*I/PVS isolate TAS03-NW4.3, Lane 3 *Sac*I/PVS isolate TAS03-NE59.3, Lane 4 = *Sac*II/PVS isolate TAS03-NW4.3, Lane 5 = *Sac*II/PVS isolate TAS03-NE59.3, Lane 6 *Ahd*I/PVS isolate TAS03-NW4.3 and Lane 7 = *Ahd*I/PVS isolate TAS03-NE59.3.

4.4 Discussion

Systemic symptom expression on *C. quinoa* in this study were mild compared to symptoms reported in other studies. Hinostroza-Orihuela (1973) was the first to report systemic infection in *C. quinoa* by PVS. Upper leaves of *C. quinoa* inoculated with PVS isolates sourced from Peruvian and Dutch cultivars, developed mosaic symptoms which developed into necrosis of petiole and veins. Necrotic symptoms were observed 18 days after inoculation, with initial systemic expression of clear yellow spots occurring on inoculated leaves at 14 days after inoculation (Hinostroza-Orihuela 1973). Fletcher (1996) observed systemic leaf mottle 7-14 days after inoculation of a New Zealand PVS isolate (PVS^{A}-NZ). Severity of symptom expression on the upper leaves of *C. quinoa* infected with different PVS isolates have been shown to vary considerably. Symptom expression on *C. quinoa* inoculated with PVS isolates of PVS^{A} from the Netherlands and West Germany ranged from mild, moderate and severe symptoms (Dolby and Jones 1987). PVS strains obtained from different Peruvian cultivars have been reported to produce systemic symptoms in *C. quinoa* (Hinostroza-Orihuela 1973). Systemic symptoms appeared after 15 day from inoculation, with different symptom types and severity observed. Hinostroza-Orihuela (1973) suggested the PVS isolate obtained from Peruvian potato cv. Mantaro was at higher titre and more infectious than isolates from other sources. Symptom expression in inoculated leaves of *C. quinoa* has been reported to vary from green spots to yellow spots between PVS isolates (Kowalska and Was 1976). No systemic infection was observed in this study with PVS isolates sourced from

different Polish and foreign cultivars. None of the PVS isolates were able to infect *L. chilense*.

Differentiation of PVS^O from PVS^A strains is currently based on the ability of the latter to produce systemic symptoms when inoculated to *C. quinoa*. However this study showed a range of symptoms and serological results. Twenty PVS isolates were characterised as PVS^O-like based on local lesions on inoculated leaves of *C. quinoa,* positive DAS-ELISA of inoculated leaves only, and absence of symptoms on inoculated leaves. A further three isolates were identified as PVS^A-like based on local lesions on inoculated leaves and mild mottling and/or chlorotic spots on non-inoculated leaves, with virus detected by DAS-ELISA in inoculated and non-inoculated leaves. Fourteen PVS isolates produced no symptoms, and inoculated leaves only, tested positive by DAS-ELISA (PVS^O-like). Four PVS isolates produced no symptoms but tested positive by DAS-ELISA in inoculated and non-inoculated leaves (PVS^A-like). Five PVS isolates produced symptoms in inoculated leaves only but tested positive by DAS-ELISA in both inoculated and non-inoculated leaves (PVS^A-like). RT-PCR-RFLP profiles of 21 isolates, including isolates identified as PVS^O and PVS^A following inoculation to *C. quinoa* produced RFLP patterns as predicted for PVS^O. However some caution is required with the results of the RT-PCR-RFLP results as there were no known PVS^A strains to test the predicted ability of *Sac*I and *Sac*II to cut the amplicon.

The inability to mechanically transmit Tasmanian PVS isolates to *L. esculentum* also provides evidence against the presence of PVS^A isolates in Tasmania. Known PVS^A strains have been shown to systemically infect *L. esculentum* with no symptom

expression, with *L. esculentum* immune to PVS^O infection (Brunt and Loebenstein 2001; Slack 1983).

This study suggests greater complexity of strain groupings of PVS may exist, and suggests both biological and molecular characterisation of isolates would be useful for characterisation. A further study of host response, host range, aphid transmissibility and phylogenetic analysis of PVS with isolates from around the world is required to define more comprehensively strains of PVS.

To the author's knowledge findings in this study were the first record of PVS infection in *S. laciniatum*. A total of 13/15 (86.6%) Tasmanian PVS isolates were shown to infect *S. laciniatum* with no symptom expression in inoculated or upper (non-inoculated) leaves. Thus *S. laciniatum* may provide a native host from PVS in Tasmania. No PVX transmission occurred with inoculation of PVS isolates co-infected with PVX. In this study PVS was not found to transmit via true seed of *S. laciniatum*, PVS has not been reported to transmit via true seed in other studies (Goth and Webb 1975). Thompson (1976) suggested *S. laciniatum* (commercial crops) should not be planted adjacent to potato crops to reduce the possibility of infection of PVX. Similarly it seems plausible that wild *S. laciniatum* growing adjacent to potato crops in Tasmania pose a potential threat for PVS as an inoculum source.

5. Mechanisms of transmission of PVX and PVS in Tasmanian seed potato

5.1 Introduction

The high incidence and prevalence of PVS reported in seed potato in Tasmania (Chapter 3) requires a greater understanding of mechanisms of viral transmission in Tasmania. The understanding of viral transmission is important to the Tasmanian potato industry for the development of effective management strategies. Primary inoculum source of mechanically transmissible viruses such as PVS and PVX, into healthy potato tuber or plants includes spread from PVS and/or PVX-infected seed tubers via handling of seed and plant-to-plant contact following sprouting of tubers and plant growth. Secondary spread of PVS and PVX can occur from virus-infected seed tubers being planted the following season (Ragsdale *et al.* 2001). Factors influencing the degree to which mechanical transmission of virus occurs includes the environment, the host plant and contact of foliage with equipment, animals and man (Franc and Banttari 2001).

In Tasmania whole seed tubers are generally cut into smaller setts (~50g). Centralised seed cutting facilities are used routinely during commercial seed production. In addition hand cutting is often conducted on early generation seed. Overseas studies have demonstrated that PVS and PVX can be transmitted between infected and healthy seed pieces on the cutting knife (Larson 1950; Franc and Banttari 1984). A number of precautions have been adopted to ensure minimal pathogen transmission

during the seed cutting operations. Precautions adopted by centralised seed cutters during seed cutting include washing seed cutting equipment at the end of the day and disinfecting equipment periodically during the cutting process. Furthermore, early generation seed crops are often cut separately from those of later generations. Some seed cutters have indicated a difficulty in disinfecting machinery more frequently during the day due to the downtime associated with sterilising, washing and allowing machinery to dry. An alternative strategy may be to disinfect tubers with antiviral chemicals soon after cutting. Antiviral chemicals such as the antiviral Viraclean (Hospital Grade 4.255 g/L benzalkonium chloride, (distributed by Whiteley Medical, Tomago, New South Wales) and Virkon® S (50% potassium peroxomonosulfate, 15% sodium alkyl benzene sulphonate and 5% sulphamic acid (distributed by United Biosciences P/L, Carindale, Queensland) applied to tubers directly after cutting might reduce PVS transmission during seed cutting operations. Viraclean is a broad range disinfestant which kills a range of bacteria, with activity against human viral pathogens. Virkon® S is a predominantly broad-spectrum antiviral chemical (Lister 2004).

The main aims of this study were: i) to determine the extent to which both mechanised and hand seed cutting contribute to virus transmission; ii) determine the ability of the antiviral disinfestants applied to cutting equipment or to tubers directly after cutting to reduce PVS transmission during seed cutting operations; iii) to determine potential means of transmission of PVS between potato plants.

5.2 Materials and methods

5.2.1 Isolates used in transmission studies

PVS and PVX isolates used in transmission experiments were obtained from seed potato crops grown in Tasmania and from Simplot Australia Pty. Ltd. Growers' Line trial (Chapter 6) (Table 5.1).

Mechanical seed cutting

Two different seed lines of cv. Russet Burbank were used for Experiment 1 in the mechanised seed cutting studies. One PVS infected potato seed line was sourced from Scottsdale (NE49). The other potato seed line was co-infected with PVS and PVX and obtained from Riana (NW24). Tubers were collected from seed potato bins maintained in cool storage for approximately 6 months (Table 5.1). Isolates used in Experiment 2 of the mechanised seed cutting studies were obtained from seed lines collected in 2003 from cool stores from Scottsdale (Jondi Cool Stores) and Latrobe (Cherry Hill Coolstores) and are listed in Table 5.1.

Hand seed cutting

For Experiment 1 of the hand seed cutting studies, tubers were sourced from two PVS infected potato seed lines sourced from Scottsdale and Riana (NE035 and NW073) (Table 5.1). PVS isolates used for hand seed cutting experiments conducted during 2005/2006 are listed in Table 5.1.

Table 5.1. Isolates of *Potato virus S* (PVS) collected from seed potato crops grown in Tasmania used in mechanised and hand seed cutting experiments.

Seed cutting experiment	Seed line code	Location of field (region/zone)[1]	Year of collection
Mechanised			
Experiment 1-2002/2003			
Crop A	NE49	Scottsdale (2)	2002
Crop B	NW24	Riana (1)	2002
Experiment 2-2003/2004			
Seed line 1	S01	Ouse (7)	2004
Seed line 2	NW02	Upper Natone (1)	2004
Seed line 3	NW03	Flowery Gully (2)	2004
Seed line 4	NW04	Riana (1)	2004
Seed line 5	NW05	Smithton (1)	2004
Seed line 6	S06	New Norfolk (7)	2004
Seed line 7	NE01	Scottsdale (3)	2004
Seed line 8	NE02	Branxholm (3)	2004
Seed line 9	NE03	Scottsdale (3)	2004
Hand cutting			
Experiment 1-2004/2005	NE035	Scottsdale (3)	2004
	NW073	Riana (2)	2004
Experiment 2-2005/2006	NWJL	Riana (2)	2005
	NW47	Sheffield (2)	2005
Experiment 3-2005/2006	NW47	Sheffield (2)	2005
	NE22	Scottsdale (3)	2005
Experiment 4-2005/2006	NWTH	Riana (2)	2005

[1] Seed growing geographical location in Tasmania shown in Figure 3.2 (Chapter 3, p. 103).

5.2.2 Mechanised seed cutting experiment 1 - 2002/2003

Two half-tonne bins of seed tubers from each of two different seed crops (Crop A and Crop B) of cv. Russet Burbank (generation 4) were used in this study. Tubers were stored at 4°C in a commercial cool-store facility, for approximately 6 months prior to the commencement of this experiment. Initial virus incidence was determined by subsampling 300 tubers from two half-tonne bins (~2500 tubers per half-tonne bin) of each crop on 12 December 2002. Tubers were stored at room temperature (15-20°) and sprouts were tested for PVS and PVX by DAS-ELISA (Appendix II) after 1-6 weeks incubation.

Seed was cut mechanically on a commercial seed cutter (Langworthy Pty. Ltd., Spreyton, Tasmania, Figure 2.4, p. 21). Cutting order of each half tonne bin of Crop A and Crop B across the seed cutter is shown in Figure 5.1. The blades of the seed cutter were washed with disinfestant using a high-pressure hose for approximately 5 minutes between Crop B bin 1 and Crop A bin 2 (Figure 5.1).

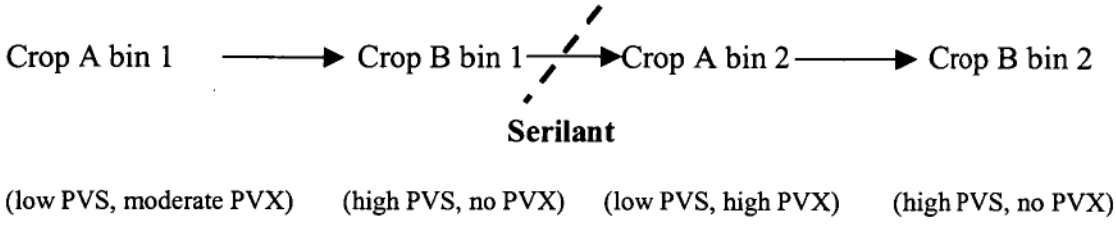


Figure 5.1. Cutting order of *Potato virus S* (PVS) and *Potato virus X* (PVX) infected seed potato tubers, cv. Russet Burbank, across a mechanised seed cutter in Tasmania.

Approximately 300 cut tubers pieces were collected as each bin of tubers passed through the cutter. Approximately 100 cut tubers were retrieved at three intervals, i) at the beginning of the cutting process, ii) midway through, and iii) toward the end of cutting process (designated as early, mid and late samples). Cut tuber pieces were stored at room temperature (15-20°C) in hessian sacks for up to 14 weeks in natural light to induce shoot formation.

Sample preparation and virus testing for whole and cut tubers

One sprout from two different eyes (approximately 0.5 cm in length) was taken from each tuber of 240 to 300 tubers. For bulked samples, sprouts were bulked into 30 lots of 10. For bulked samples the virus incidence was then estimated by the method of Gibbs and Gower (1960) (see Section 3.2.4, pp. 101-102).

In addition individual virus testing by DAS-ELISA (Appendix II) was conducted on sprouts of whole pieces 3 weeks after the cutting process. Two sprouts from each tuber were used in each test. The numbers of tubers tested in this manner varied from 20 (Crop B bin 2) to 290 (Crop A bin 2). The disparity of sample numbers was due to reliance on seed cutters to provide tuber samples. All cut seed pieces were virus tested for PVS and PVX by DAS-ELISA (Appendix II) at 14 weeks after cutting process.

5.2.3 Mechanised seed cutting experiment 2 - 2003/2004

A further assessment of virus transmission on centralised seed cutters was undertaken in 2004. Tuber samples were collected from a half tonne bin prior to cutting and a

further sample immediately after cutting. A total of 9 seed lines were collected from Cherry Hill Cool Store, Latrobe and 6 seed lines from Jondi Cool Store, Scottsdale. Tubers were removed out of cool storage between 13-20 October 2004. Tuber samples were collected on 27, 28, 29 October 2004 and 3 November 2004. Due to high virus levels in whole tubers virus tested, only 9/15 lines with low virus were subsequently assessed for virus spread.

Between 45-50 seed pieces were collected from each of seed line lines collected from Latrobe (NW). Eighty seed pieces per seed line were collected from Scottsdale (NE). A sample of between 34-50 whole tubers (pre-cut sample) and between 25-82 post cutting (cut seed pieces) were labelled and stored at room temperature for 1 to 2 weeks. Tubers were collected by staff at each centralised seed cutter, and despite requests for samples between 75 and 100 for both pre-cut and post-cut seed pieces tuber sample numbers varied due to reliance on seed cutters. Sprouts from whole tubers (pre-cut) were tested for PVS and PVX by DAS-ELISA (Appendix II). Lines with 100% PVS infection were not tested further, all other seed pieces were planted out for later testing (two lines were discarded due to 100% PVS infection, not listed).

Cut seed tuber pieces were stored at room temperature in the laboratory (15-20°C) and on 16-17 November 2004 (13-21 days after collection) planted into 200 mm pots in commercial potting mix in an aphid proof tent. Each pot also had the addition of a clear plastic tree guard to prevent plant to plant contact. Leaflets were collected 8 weeks after planting, and tested by DAS-ELISA (Appendix II) for PVS and PVX.

5.2.4 Hand seed cutting Experiment 1- 2004/2005, transmission of virus during seed cutting and impact of treatments

Tubers of cv. Russet Burbank were used to assess a range of treatments for disinfecting cutting equipment during hand seed cutting operations. In addition potential differences in viral transmission on different potato cultivars (cvs. Kennebec, Ranger Russet and Shepody) were also assessed during the seed cutting experiments.

Experiment 1 was conducted on 8-9 September 2004 (PVS seed line from crop 2, NW073) and 15-16 September 2004 (PVS seed line from crop 1, NE035) with individual box cutter metal blades (length:120mm, width:20 mm, thickness:0.5 mm). Initially a cut was made through a PVS infected seed piece with a metal blade followed by a cut through a healthy seed piece. This was followed by the application of different treatments (Table 5.2) applied to the blades. Using individual blades this process was repeated for each of twenty replicates of each treatment. All blades were washed and autoclaved prior to use. PVS infected tubers used as source seed pieces were virus tested for PVS by DAS-ELISA (Appendix II) prior to use in Experiment 1. Prior to cutting hand seed cutting experiment 1 virus testing for PVS and PVX (by DAS-ELISA, Appendix II) was conducted on sprouts from 20 tubers of 'healthy' (virus free) seed (cvs. Russet Burbank, Kennebec, Shepody and Ranger Russet) and found to be PVS- and PVX-free.

Treatments included the antiviral agents 1% Virkon® S and 0.1% Sodium hypochlorite (bleach), and methylated spirits which are used during seed cutting to reduce the transmission of PVS. Methylated spirits has been utilised as an

antibacterial agent at 70% and was tested as a potential antiviral agent. In addition Virkon® S was also assessed for possible physiologically effects on potato tissue and included a treatment of applying Virkon® S as a 1% solution after cutting. Thermal inactivation point of PVS is approximately 55-60°C (Hinostroza-Orihuela 1973; Banttari *et al.* 1993), thus 100°C of heat applied to blades for 15 seconds was tested. Cement is used sporadically as a seed dust dressing in seed potato production in Australia (Wilson *et al.* 1999). Cement dust applied to the cut tuber surface aids in curing the cut surface. The full set of treatments used in this study are listed in Table 5.2.

Table 5.2. Different treatments used for hand seed cutting Experiment 1 during 2004/2005 to assess transmission of *Potato virus S* (PVS) sourced from seed potato in Tasmania.

Treatment No.	Treatment Description[1,2]
1	Control - Cut infected - No sterilant – cut healthy
2	Cut infected - No sterilant, place blade in incubator (24 hours/20°C)– cut healthy
3	Cut infected - No sterilant– cut healthy – dip seed piece in 1% Virkon® S
4	Cut infected - No sterilant 24 hours – cut healthy – place cut surface of tuber in Virkon® S powder and cement dust (5g Virkon® S/500g cement)
5	Cut infected – place blade in 0.1% a.i. sodium hypochlorite for 1 min - rinse in water – cut healthy
6	Cut infected – place blade in 0.1% a.i. sodium hypochlorite for 10 min - rinse in water – cut healthy
7	Cut infected – place blade in 1% Virkon® S for 1 min – rinse in water – cut healthy
8	Cut infected - place blade in 1% Virkon® S for 10 min – rinse in water – cut healthy
9	Cut infected – place blade in 70% methylated spirits for 1 minute and allow to evaporate for 5 minutes – cut healthy
10	Cut infected – Dip blade in 70% methylated spirits 10 minutes and allow to evaporate 5 minutes - cut.
11	Cut infected - Dip blade in 70% methylated spirits and flame over bunsen and cool 5 minutes – cut healthy
12	Cut infected – hold blade in front of heat gun for 15 seconds (100°C) and cool 5 minutes – cut healthy
13	Cut infected - No sterilant – cut healthy Shepody
14	Negative control - Cut healthy (cv. Russet Burbank) with clean blade and plant
15	Cut infected - No sterilant – cut healthy Ranger Russet
16	Cut infected - No sterilant – cut healthy Kennebec
17	Cut infected - No sterilant – cut healthy Russet Burbank and coat and dust with cement (using PVS seed line NW073 only)

[1] Treatments 1-12 applied to blade except for treatment 3 which was applied to the cut surface of the tuber.
[2] Treatments 1-12, 14 and 17 conducted on cv. Russet Burbank.

After tuber pieces were cut and treatments applied, one seed piece was discarded, and the other placed in a labelled plastic tray and stored at room temperature (15-20°C) for 2 days prior to planting. Seed pieces were planted in commercial potting mix in 150 mm pots on 15-16 September 2004 (PVS NE035 seed line) and 23-24 September (PVS NW075 seed line). Clear plastic bags (300 x 200 mm) were placed around each pot to prevent contact between emerging foliage of growing plants. Several holes were cut at the base of each plastic bag to allow for drainage. Pots were placed in an aphid proof enclosure (Figure 5.2). Individual leaflets from each plant were collected from at 82 DAP (NE035) and 63 DAP (NW075) and virus tested for PVS by DAS-ELISA (Appendix II). During collection of samples, a plastic bag was placed over each leaflet and the leaflet pinched off in the bag to avoid potential virus transmission.

Figure 5.2. Potato plants from hand seed cutting experiment (2004/2005) in opened aphid proof enclosure showing plants with plastic bags separating foliage.

5.2.5 Hand seed cutting Experiment 2 - 2005/2006, effect of disinfestation after hand cutting on virus transmission and on growth of plants

Inoculum used in this study was obtained from highly infected tubers of cv. Ranger Russet (PVS seed line NW47). Twenty PVS-infected tubers were used as a source of PVS virus. For each of the following treatments (except treatment 7) individual knives were sliced through each of the 20 infected tubers and immediately sliced through 20 virus-free minitubers. Treatments used in hand seed cutting Experiment 2 are listed in Table 5.3.

Table 5.3. Different treatments used for Experiment 2 (hand seed cutting) to assess the effect of disinfestation of tubers after hand cutting of seed potato from Tasmania.

Treatment No.	Treatment Description
1	Inoculated and cut minitubers dipped in 1% Virkon® S solution (w/v).
2	Inoculated and cut minitubers dipped in 5% Virkon® S solution (w/v).
3	Inoculated and cut minitubers dipped in undiluted Viraclean solution.
4	Inoculated and cut minitubers rolled in cement containing 1% (w/w) Virkon® S powder.
5	Inoculated and cut minitubers rolled in cement only after cutting
6	Inoculated and cut mintubers with no further treatment (positive control).
7	Non-inoculated and cut mintubers with no further treatment (negative control).

Mintubers were cut and treated on 18 November 2005. Cut mintubers were dipped in sterilants for 2 minutes after cutting and 5- 10 seconds duration. Minitubers were dried on the bench and half of each was planted in commercial potting mix in 200 mm

diameter pots on 25 November 2005 and grown in a greenhouse. Leaflets were collected on 23 January 2006 (59 DAP) and virus tested for PVS by DAS-ELISA (Appendix II). To avoid potential virus transmission during collection of samples, a plastic bag was placed over each leaflet and the leaflet pinched off in the bag. At senescence on 1 May 2006, tubers were collected and placed in cool store (4°C). Two tubers from each treatment were replanted into single pots (1 L) on 5 August 2006 and grown on for 7 weeks. One leaflet from each stolon was collected on 23 October 2006 and a subsample comprising part of each leaflet tested for PVS by DAS-ELISA (Appendix II).

5.2.6. Hand seed cutting Experiment 3 - 2005/2006, effect of disinfestation of tubers after hand cutting on subsequent plant growth

Tubers were obtained from a highly infected seed lot cv. Ranger Russet (PVS isolate NWJL) obtained from Riana. Whole tubers were sprouted, and sprouts were tested for PVS infection by DAS-ELISA (Appendix II). Fifteen PVS-infected tubers were selected as PVS source tubers.

Healthy tubers (cv. Russet Burbank) were obtained from a seed lot harvested from a field in which virus had not been detected during routine DPIW seed certification survey testing in 2004/2005. Tubers were sprouted and tested for PVS infection by DAS-ELISA (Appendix II) prior to the experiment. Healthy tubers were treated on 24 November 2005 as listed in Table 5.4., with 15 replicates per treatment.

Table 5.4. Different treatments used for hand seed cutting Experiment 3 to assess the effect of disinfestation of tubers after hand cutting seed potato from Tasmania.

Treatment	Treatment Description
Negative control:	Cut healthy tubers only
Positive control:	Cut infected tuber followed by healthy tuber
Virkon® S 0.1% (w/v):	Cut infected tuber, followed by healthy tubers then dipped in Virkon® S 0.1% (0.5g Virkon® S /500 ml distilled water).
Viraclean 10% (w/v):	Cut infected tuber followed by healthy tuber then dipped in Viraclean 10% (50 ml Viraclean and 450 ml distilled water).

One half of each cut tuber was discarded. For disinfestant treatments, the remaining half was dipped in sterilant for up to 10 seconds duration, no longer than 2 minutes after cutting. Tubers were dried on the bench overnight prior to planting in commercial potting mix in 200 mm pots on 25 November 2005. Plants were maintained in the greenhouse for 3 months. One leaflet from each plant was collected on 2 March 2006 and tested for PVS by DAS-ELISA (Appendix II). To avoid potential virus transmission during collection of samples, a plastic bag was placed over each leaflet and the leaflet pinched off in the bag. Tubers were collected from senescencing plants on 1 May 2006 and stored (4°C). Two tubers from each treatment were planted in single pots (1 L) in commercial potting mix on 2 October 2006, grown on for approximately 6 weeks and 1 leaflet collected from each plant on 21 November 2006. Leaflets were tested for PVS by DAS-ELISA (Appendix II).

5.2.7. Transmission of PVS between potato plants

An experiment was established in which plants were either placed in aphis proof cages or left uncaged and furthermore were either placed in contact with PVS-infected plants or not in contact. The possible outcomes are given in Table 5.5.

Table 5.5. Conclusions that could be drawn if there had been transmission (+) or no transmission (-) to initially healthy plants in particular treatment combinations.

	With infected plants	Without infected plants
Aphid proof caged plants	+[1] = mechanical	+ = contamination
	-[2] = no mechanical	- = no contamination
Not caged	+ = mechanical/aphid	+ = aphid transmission
(aphids not excluded)	- = no mechanical/aphid	- = no aphid transmission

[1] = PVS transmission occurred

[2] = no PVS transmission occurred

Healthy Ranger Russet minitubers obtained from DPIW were planted into 20 mm pots in commercial potting mix on 14 December 2005 and grown under aphid proof conditions. Virus source tubers were obtained from a G2 crop identified in the 2004/2005 growing season as having high PVS infection. Virus source tubers were maintained in cool storage for 5 months, then placed in labelled containers on the laboratory bench to encourage sprout formation. Sprouts were subsequently tested for PVS by DAS-ELISA (Appendix II). PVS-infected tubers were subsequently planted into pots (as above) and used as source plants in a PVS transmission potato plant trial.

The trial was established on 22 December 2005 and included four treatments: i) twenty healthy potato plants placed in an aphid proof caged without; ii) twenty healthy potato plants placed in an aphid proof caged with five PVS-infected potato plants; iii) twenty healthy potato plants placed in a group within a non-aphid proof greenhouse (non-caged) without inoculum, and iv) twenty healthy potato plants placed in a group within a non-aphid proof greenhouse (non-caged) with five PVS-infected potato plants. For each treatment, 20 healthy plants were arranged in two rows of 10 plants, placed approximately 20cm apart. Where required, 5 virus source plants were placed between the two rows, so that each source plant was in contact with 4 adjacent healthy plants. Abrasion between plants to enable potential mechanical inoculation of PVS between PVS-infected source plants and healthy potato plants was facilitated by large evaporative cooling fans fitted to the greenhouse wall in close proximity to this trial.

All four treatments were located in the same greenhouse and separated by a distance of approximately 6 metres. Uncaged plants were exposed to naturally occurring populations of aphids in the greenhouse. Alatae (winged) aphids flights were monitored using yellow sticky aphid traps (9.5 cm wide x 23.0 cm long) placed above and near the trial. In addition plants were observed frequently for colonising aphid populations and any aphids present were preserved in 70% alcohol until processing and identification. Traps were stored at 10°C until forwarded to C. Young (DPIW) for processing and identification. Aphids located under a dissecting microscope (50 X) were removed from the stick traps by soaking in a dipentine-based solvent (DeSolvit™, RCR International, Victoria, Australia). Species keys were used to

identify aphids (Blackman and Eastop 2000), in addition to comparison with type specimens held in the insect collection at the DPIW, New Town, Tasmania.

Leaflets were collected from plants nearing senescence on 2 March 2006 and tested for PVS by DAS-ELISA (Appendix II). To avoid potential virus transmission during collection of samples, individual leaflets were collected by placing an individual plastic bag over each leaflet and pinching leaflet off in the bag. Tubers were collected from each plant on 1 May 2006, placed in paper bags and maintained at 4°C for 5 months. Two tubers from each plant were replanted into single pots in commercial potting mix on 2 October 2006, and grown under greenhouse conditions. After 6 weeks (21 November 2006) leaflets were collected and tested PVS by DAS-ELISA (Appendix II).

5.3 Results

5.3.1 Mechanised seed cutting Experiment 1 - 2002/2003

Results from this trial were not conclusive. There was a slight increase in PVS within Crop A bin 1 and Crop A bin 2 when whole tubers were cut (Table 5.6). However, this increase may have been within the bounds of natural variation in virus incidence between samples. Similarly, there was no clear evidence of transmission of PVS between Crop B, bin 1 (with high incidence of PVS) and Crop A, bin 2 (with low incidence of PVS). There was a large apparent increase in the incidence of PVX within Crop A bin 1 seed (Table 5.6). However prior to cutting, there was considerable variation between virus testing of bulked samples compared to individual samples, which called into question the increase noted after cutting. Furthermore, there was an inexplicable decrease in PVX incidence after cutting the seed in Crop A bin 2 (Table 5.6). There was no evidence of transmission of PVX between the highly infected Crop A and the uninfected Crop B (Table 5.6).

Table 5.6. Virus incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) in bulked samples[1] and in individual samples[2] before and after seed cutting.

	Order of seed cutting:			
	Crop A bin 1	**Crop B bin 1**	**Crop A bin 2**	**Crop B bin 2**
Potato virus S				
Incidence before cutting:				
Bulked samples[1]	5/30 (1.3%)	30/30 (>50%)[3]	9/30 (2.5%)	30/30 (>50%)[3]
Individual samples[2]	1/85 (1.2%)	198/198 (100%)	17/290 (5.9%)	20/20 (100%)
Incidence after cutting:				
Bulked samples[1]	-	-	-	-
Individual samples[2]	13/300 (4.3%)	300/300 (100%)	20/240 (8.3%)	299/300 (99.7%)
Potato virus X				
Incidence before cutting:				
Bulked samples[1]	19/30 (6.2%)	-	30/30 (>50%)[3]	-
Individual samples[2]	23/85 (27.1%)	0/30 (0%)	9/20 (45%)	0/30 (0%)
Incidence after cutting:				
Bulked samples[1]	-	-	-	-
Individual samples[2]	120/300 (40%)	0/300 (0.0%)	52/240 (21.7%)	0/300 (0%)

[1] Bulked samples tested as 30 samples of 10 tubers.
[2] Individual tubers/seed pieces tested.
[3] Caution is required with mean estimation incidence. As previously mentioned, cases where the Gibbs and Gower (1960) formula gave an estimate of 100% infection were in the range of 40-100% when individual leaves were retested.

5.3.2 Mechanised seed cutting experiment 2 - 2004/2005

Results of virus transmission during seed cutting from this trial were variable. In 5 seed lines (lines 1, 2, 4, 5 and 6), moderate to large increases in PVS infection occurred between pre-cutting and post cutting (Table 5.7). However, in other seed lines, PVS incidence was similar (lines 3, 8) or declined (lines 7, 9) between pre-cutting and post cutting. Most crops were free of detectable PVX (Table 5.7). In seed line 4, PVX was detected only after cutting. Conversely, PVX was detected in seed line 5 prior to cutting, but not post cutting. A paired t-test conducted on PVS incidence was non significant (P=0.395) suggesting no difference between incidence pre-and post-cutting.

Table 5.7. Incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) within pre- and post–cut potato seed pieces after cutting on a mechanised seed cutter located at a commercial cool store in Tasmania. Seed was initially obtained from seed potato growers of Tasmania during 2003/2004 potato growing season.

Seed Line No.	Location[1]	Total tubers tested (Pre/Post cut)	PVS incidence (%)		PVX incidence (%)	
			Pre cut	Post cut	Pre cut	Post cut
1	Ouse (S)	42/28	16.7	64.3	0	0
2	Upper Natone (NW)	38/25	26.3	36.0	0	0
3	Flowery Gully (NW)	46/82	19.6	14.6	0	0
4	Riana (NW)	40/76	30.0	44.7	0	6.6
5	Smithton (NW)	47/58	27.7	63.8	10.6	0
6	New Norfolk (S)	50/72	14.0	25.0	0	0
7	Scottsdale (NE)	37/36	89.2	77.8	0	0
8	Branxholm (NE)	34/39	88.2	82.1	0	0
9	Scottsdale (NE)	39/56	97.4	66.1	0	0

[1]NWC – North West Coast, NE = North East, S = South

Aphids were detected in the tents at 42 DAP and were collected and identified. The aphids sampled were all *Aulacorthum solani* (glasshouse-potato aphid, foxglove aphid) which is reportedly not a vector of PVS (Wadrop *et al.* 1998).

5.3.3 Hand seed cutting Experiment 1 - 2004/2005, transmission of virus during seed cutting and impact of treatments

No PVS was detected in the negative control for Russet Burbank (treatment 14) following planting (Table 5.8), or in sprouts tested prior to planting confirming that healthy tubers were free of virus and that opportunity for contamination in the experiment was limited. However, no transmission of PVS was detected in the positive control for Russet Burbank (treatment 1), in which knives were passed through infected tubers prior to healthy tubers (Table 5.8). This is despite transmission of PVS occurring for Shepody, Ranger Russet and Kennebec (treatments 13, 15 and 16 respectively). In Kennebec, transmission occurred to 35% of tubers with both isolates (Table 5.8). The lack of any transmission of PVS in the positive control for Russet Burbank prevented the statistical comparison of differences between sterilant treatments or varieties. However, as transmission of PVS occurred in some of the sterilant treatments, some conclusions could be obtained as to the efficacy of some treatments. Results suggested that heating the cutting blade (treatment 12), or exposure of the cutting blade for 1 or 10 minutes to 0.1% a.i. sodium hypochlorite (treatments 5 and 6), 1% Virkon S (treatments 7 and 8) or 70% methylated spirits (treatments 9, 10 and 11) were ineffective in completely preventing transmission of PVS. Similarly dusting the cut surface with cement as was traditionally used commercially to assist in curing the cut surface was also ineffective in completely preventing PVS transmission.

PVS isolate NW073 appeared to be more transmissible by cutting than NE035, with transmission in 8/12 and 4/12 treatments respectively in those treatments which involved Russet Burbank and both virus isolates. Similarly across all treatments involving Russet Burbank and both isolates, there was transmission in 41/240 and 5/240 tubers for isolate NW073 and NE035 respectively. However, again the lack of transmission in the positive control for Russet Burbank precluded statistical analysis (Table 5.8).

Table 5.8. Virus incidence for *Potato virus S* (PVS) with different treatments used during hand seed cutting.

Treatment no. and description[1]	PVS seed line 1- NE035 PVS	PVS seed line 2- NW073 PVS
1. Control - Cut infected - No sterilant – cut healthy	0	0
2. Cut infected - No sterilant 24 hours/20°C incubator – cut healthy	0	0
3. Cut infected - No sterilant– cut healthy – dip seed piece in 1% Virkon® S	0	0
4. Cut infected - No sterilant 24 hours – cut healthy- place cut surface on Virkon® S powder and cement dust (5g Virkon® S/500g cement)	0	0
5. Cut infected - 0.1% a.i. sodium hypochlorite 1 min – rinse in water – cut healthy	1/20	3/20
6. Cut infected - 0.1% a.i. sodium hypochlorite 10 min – rinse in water – cut healthy	0	5/20
7. Cut infected - 1% Virkon® S 1 min – rinse in water – cut healthy	1/20	4/20
8. Cut infected - 1% Virkon® S 10 min – rinse in water – cut healthy	0	6/20
9. Cut infected - Dip in 70% methylated spirits 1 minute and allow to evaporate 5 minutes – cut healthy	2/20	5/20
10. Cut infected - Dip in 70% methylated spirits 10 minutes and allow to evaporate 5 minutes - cut.	1/20	3/20
11. Cut infected - Dip in 70% methylated spirits and flame over bunsen and cool 5 minutes – cut healthy	0	5/20
12. Cut infected – Heat gun for 15 seconds (100°C) and cool 5 minutes – cut healthy	0	6/20
13. Cut infected - No sterilant – cut healthy Shepody	1/20	5/20
14. Negative control - Cut healthy Russet Burbank with clean blade and plant	0	0
15. Cut infected - No sterilant – cut healthy Ranger Russet	0	2/20
16. Cut infected - No sterilant – cut healthy Kennebec	7/20	7/20
17. Cut infected - No sterilant – cut healthy Russet Burbank and dust with cement	-	4/20

[1] Treatments 1-12, 14 and 17 were conducted on cv. Russet Burbank.

5.3.4. Hand seed cutting Experiment 2 - 2005/2006, effect of disinfestation after hand cutting on virus transmission and on growth of plants

No virus was detected by DAS-ELISA in any leaf samples collected prior to senescence or from leaflets obtained from tubers grown on in the following season indicating no transmission of PVS. Some bleaching and retardation of sprout growth was noted with 5% Virkon® S solution and with undiluted Viraclean solution. Other treatments appeared unaffected. Treatment with undiluted Viraclean appeared to reduce emergence, with only 14/20 tubers developing into plants. In all other treatments more than 17/20 tubers planted developed into plants. However, there was no observable difference in plant growth between treatments at 6 weeks after treatment.

5.3.5. Hand seed cutting Experiment 3 – 2005/2006, effect of disinfestation of tubers after hand cutting on subsequent plant growth

There was no observable difference between treatments on emergence with 11,14 11 and 13 of 15 tubers producing plants for the negative control, positive control, Viraclean and Virkon® S treatments respectively. Similarly there were no significant differences between treatments in the number of tubers, average tuber weight total weight of tubers per plant (Table 5.9).

Table 5.9. Effect of various treatments on average number of tubers, average tuber weight and total weight of tubers per plant.

Treatment	Number of tubers	Average tuber weight (g)	Total weight of tubers (g)
Control (-ve)	5.4	47.6	248.1
Control (+ve)	5.3	55.0	277.6
Viraclean (10%)	5.6	48.6	239.7
Virkon® S (1%)	6.1	50.5	283.2
P =	0.68 (ns)	0.65 (ns)	0.11 (ns)

5.3.6. Transmission of PVS between potato plants

PVS was not detected by DAS-ELISA in leaflets collected from plants grown from minitubers just prior to senescence. However, virus was detected in leaflet samples from all virus source plants at this time.

In plants collected from tubers grown the following season, PVS was not detected in plants subjected to either caged or non-caged treatments in which there were no infected source plants (Table 5.10). However, there was a high incidence of PVS in plants that had been exposed to infected source plants in both caged and non-caged treatments (Table 5.10). Some aphids were noted on both caged and uncaged potato plants were identified as *A. solani* (glasshouse-potato aphid, foxglove aphid), not a known vector for PVS (Wardrop *et al.* 1998). This indicated mechanical transmission occurred between plants but gave no evidence of aphid transmission (Table 5.10).

Table 5.10. The incidence of *Potato virus S* (PVS) in foliar samples collected from potato plants maintained in aphid proof cages or non-caged and exposed to contact with infected source plants (+) or not exposed (-).

	Number of PVS infected/number tested on:	
Treatment	**02/03/2006**[a]	**21/11/2006**[b]
Caged (-)	0/20	0/20
Caged (+)	0/20	18/20
Non-caged (-)	0/20	0/20
Non-caged (+)	0/20	16/20

Leaflets were collected and tested from plants prior to senescence[a] and from tubers collected from plants and grown on the following season[b].

5.4 Discussion

The role of mechanised and hand seed cutting of potato in transmission of PVS and PVX in Tasmania was investigated. Results from these studies show limited transmission of PVS during seed cutting experiments. Results of mechanised seed cutting experiments 1 and 2 were variable. No evidence of PVS or PVX transmission was observed during mechanised seed cutting experiments 1, with a slight increase in PVS probably within the bounds of sampling error. For mechanised seed cutting experiments 2, although the majority of crops showed an increase in PVS after cutting, the decline in incidence in some crops calls into question the veracity of this result. While seed cutting is known to spread PVS and PVX, this trial failed to demonstrate conclusively that seed cutting is a source of virus spread in Tasmania. Furthermore low sample numbers obtained from the seed cutting experiments and experimental design are likely to have contributed to a large sampling error in this trial.

Transmission studies of PVS during hand seed cutting demonstrated limited virus transmission in experiment 1 and no transmission in experiment 2, from infected to uninfected tubers by hand-cutting. The low transmission of PVS by seed cutting is at odds with results of overseas studies. Although low virus transmission of PVS occurred in this trial this may also be a result of experimental design. In addition no virus transmission occurred in positive controls of seed cutting experiment 1. Franc and Banttari (1984) demonstrated that transmission of PVS from infected to uninfected tubers by hand cutting in cv. Russet Burbank, increase significantly if the cutting knife passed through a sprout of a tuber (45.2%) compared to knife contact

with non-sprout tuber tissue (24.5%). Franc and Banttari (1984) also demonstrated that transmission of PVS by seed cutting had different transmission efficiency between cultivars and demonstrated that indexing and sanitation procedures are necessary during the production of PVS-free potato seed. For example a significant increase (P=0.01) of 76.7% and 62.6% in infected plants for cvs. Russet Burbank and Kennebec, respectively, after cutting infected tubers followed by healthy tubers compared to cv. Norland (25% infection). Greater numbers of plants were infected when cutting occurred through sprouts than non-sprouted material (cv. Kennebec) (Franc and Banttari 1984). Low transmission in seed cutting experiemnts conducted in our studies may also be attributed in some cases to the use of non-sprouted material.

Spread of PVS on contaminated seed-cutting machine was also demonstrated for the cv. Norgold Russet (Wright 1987). A seed line with 0.0-1.5% incidence of PVS was passed through a seed cutter after cleaning or after cutting a line with 20% PVS on the same day. Seed cut on the cleaned machine remained at between 0.0-1.5% incidence, while in two lots cut on the contaminated machine the incidence of PVS increased to 1.9-7.2% and ± 15%, respectively. PVS replication within the tuber is induced when potato tubers are mechanically wounded (Morelli and Vayda 1996). Poor handling of tubers prior to cutting might therefore stimulate PVS replication and increase virus transmission between tubers during the cutting procedure.

PVX has been shown to be transmitted readily during seed cutting. Larson (1950) showed a ringspot strain of PVX to be transmitted with greater frequency by the cutting knife when virus infected source tubers were cut through the eyes (52%) compared with cuts through source tubers that avoid eyes (24%). However there was

no evidence for transmission of PVX on a commercial seed cutter in mechanised seed cutting experiment 1.

Results from this study suggests that strains of PVS within Tasmania may not be as readily mechanically transmissible by seed cutting as those overseas or that transmission of PVS requires transmission of significant amounts of infected sap, e.g. on a mechanical seed cutter. Therefore while several studies have implicated seed cutting in spread of PVS, the contribution of seed cutting to overall virus levels in Tasmania is yet to be determined.

The lack of virus transmission in these experiments prevents an assessment of the ability of disinfestants to reduce virus transmission during seed cutting. However, some transmission of PVS was noted in some of the sterilant treatments. Fletcher *et al.* (2004) demonstrated that exposure of blades treated with PVX to concentrations of Virkon® S (0.1 and 1.0%), similar to that used in this experiment for a period of 30 seconds was able to significantly reduce but not eliminate subsequent transmission of PVX to indicator plants.

Disinfectants applied to tubers would be active on the cut surface of the tuber for somewhat longer than this, and would permeate into the surface layers, which would further indicate potential for eradication of virus from the cut surface.

Some damage to sprouts of dipped seed tubers was evident following treatment with concentrated Viraclean and Virkon® S 5% w/v, but not Virkon® S 1% w/v. However,

there were no obvious differences in plant growth or in subsequent tuber number per plant, total weight of tubers per plant or average tuber weight in these experiments. Treatments of tubers with disinfectants after cutting for control of virus transmission could potentially be a viable strategy if it could be demonstrated that there was no subsequent phytotoxicity effect and that non-phytotoxic concentrations could reduce virus transmission. Due to the lack of transmission in these experiments this latter point could not be ascertained. Further larger scale field trials are required to assess phytotoxicity and ability to reduce virus transmission. Other disinfestants should be examined for their ability to reduce virus transmission of PVS and PVX during seed cutting. Fletcher *et al.* (2004) studied the ability of several disinfestants to reduce transmission of PVX and Tobacco mosaic virus on knives (Table 5.11).

Table 5.11. Effect of dipping blades contaminated with *Potato virus X* (PVX) or *Tobacco mosaic virus* (TMV) in disinfestants for 30 seconds subsequent ability to inoculate indicator plants by cutting (from Fletcher *et al.* 2004).

Disinfestant	Concentration (% a.i)	% virus transmission to indicator plants	
		PVX	TMV
Sodium hypochlorite[1]	0.24%	10	0
	2.4%	0	10
Benzoic acid[2]	1%	0	100
	5%	0	90
Hydrogen peroxide[3]	15	40	90
	10%	0	80
Chitosan	0.01%	11.1	100
	0.1%	0	90
Potassium peroxomonosulfate[4]	0.1%	20	100
	1%	10	40
Didecylmethyl ammonium chloride[5]	1%	30	100
	10%	20	80
Water control	-	40	100

[1] Dynawhite 94.8% sodium hypochlorite)

[2] Culticlean (9% benzoic acid)

[3] Geosil (25% hydrogen peroxide and silver)

[4] Virkon® S (50% potassium peroxomonosulfate and 15% sodium alkyl benzene)

[5] Sporekill (12% didecylmethyl ammonium chloride)

Some of these disinfestants have low phytotoxicity at rates which are virucidal. Benzoic acid has been used at low concentrations within recirculating hydroponic systems and sprayed onto plants without phytotoxicity. Similarly, concentrations of Virkon® S below 1% are generally considered to be not phytotoxic to a variety of plants. Chitosan has been used as a postharvest treatment of carrot at 2 or 4% for

control of Sclerotinia rot without evidence of phytotoxicity (Cheah *et al.* 1997) and for control of powdery mildew when sprayed onto plants. Therefore, there would appear to be opportunities for developing protocols for treating tubers with disinfectants to reduce transmission of virus and other pathogens during seed cutting.

The findings that plants grown from healthy mintubers become infected in both caged and non caged treatments and only in those treatments exposed to infected source plants was suggestive of mechanical transmission between plants. In this trial primary infections of PVS could not be detected by ELISA within the season. This may have been due to transmission occurring late in the season, which did not allow virus sufficient time to increase to levels detectable by ELISA. The lack of transmission to non-caged plants without infected source plants provides no evidence of virus transmission by aphids in this trial. However known vector species were not present. The high amount of transmission to healthy plants grown in close contact with PVS-source plants is interesting given that plants in this trial would have been exposed to potentially less wind-rub and damage than field-grown plants. It should be noted that plants were placed on a geotextile irrigator mat, which may have permitted root contact between plants later in the season. Therefore the possibility of transmission between roots rather than foliage cannot be ruled out. Nevertheless the experiment indicated close contact between plants facilitates transmission.

6. Effect of PVS and PVX on yield of Russet Burbank seed potato

6.1 Introduction

Recent finding of PVS and PVX in Tasmanian seed potato crops (Chapter 3) is of concern to the industry due to possible problems of achieving certification, barriers to the sale of seed interstate and the effects of these viruses on yield. Generally it is considered that PVS and PVX have a relatively mild effect on potato growth and yield. Overseas studies have shown yield reductions of up to 10-20% when these viruses occur singly, or up to 40% when co-infection occurs (Stevenson *et al.* 2001). Differences in the magnitude of yield reduction between studies have been attributed to different strains of virus, geographic locality of the study and the cultivar of potato. However, there is no data on the effect of local strains of these viruses in Tasmania and limited data is available from other parts of Australia.

This study capitalised on the "Grower's Lines Trial" conducted annually by Simplot Australia Pty. Ltd. in which seed is sourced from Simplot growers and planted in replicated plots in one locality. The relative performance of seed lines is assessed and factors associated with lines that perform well and those that perform poorly are identified. Information from these trials is integrated into the agronomic advice given to growers to improve seed potato quality. The measurement of the effect of virus on yield is fundamental to assessing the cost effectiveness of virus management practices. Reliable estimates of yield loss can only be obtained by conducting dedicated trials over multiple localities and years. However this was not possible given the time and

financial constraints of this study. Due to the fact potato seed may also have different incidence of virus, this trial afforded the opportunity to investigate the effects of local strains of PVS and PVX on yield and quality of the processing potato cultivar Russet Burbank under Tasmanian conditions.

This study was conducted over three consecutive potato growing seasons with the main aims of: i) assessing the effects of PVS and PVX on yield of Russet Burbank from Simplot Growers' Line trials; ii) comparing PVS and PVX incidence of infected plants grown from seed sourced from different seed potato growing regions of Tasmania, and iii) determining the ability to detect late season infection of PVS and PVX in Russet Burbank.

6.2 Materials and methods

6.2.1 Field site and data collection for yield analyses

Trials were established at Riana, Tasmania and maintained by Simplot Australia Pty. Ltd. over three consecutive potato growing seasons 2002/2003 (2002), 2003/2004 (2003) and 2004/2005 (2004) and used to investigate the relationships between virus incidence and yield. Trials consisted of cv. Russet Burbank with seed tubers sourced from Simplot Australia Pty. Ltd. seed growers. Seed lines assessed for virus incidence of PVS and PVX from different seed potato growing regions of Tasmania are listed (Table 6.1). Staff from Simplot Australia Pty. Ltd conducted all planting, agronomic activities, harvest and yield assessment.

Trials were planted on 18 November 2002, 27 November 2003 and 21 October 2004. Trial planting consisted of a sample of seed from each of 60, 56 and 52 seed lines of Russet Burbank sourced from tubers harvested from single fields for the 2002, 2003 and 2004 seasons, respectively. A 50 kg composite sample of seed tubers was obtained from each seed line, consisting of 5 kg from each of eight half-tonne bins stored in a commercial cool-store after harvest. Seed was randomly selected without regard to size or appearance.

Each trial was arranged as a randomised block design with plots of the 60, 56 and 52 seed lines arranged in three replicate blocks for the 2002, 2003 and 2004 seasons, respectively. Blocks were separated by four unplanted rows. Each replicate plot consisted of two rows of potato, 10 m long. Seed was hand-cut using knives sterilised with methylated spirits or bleach between lines. Seed was planted at 30 cm spacing

using a Faun planter. Fertiliser was band applied with the seed at a rate of 175 kg N/ha, 240 kg P/ha and 220 kg K/ha plus trace elements. Other aspects of crop agronomy (irrigation and pesticides) were conducted as per commercial practice. Irrigation water was applied using a linear move irrigator as required.

Observations of plant health and growth were made on the central 5 m of row during the season. Trials were harvested on 10 April 2003, 25 March 2004 and 12 May 2005. Tubers from the central 5 m of the rows were harvested with a 2-row lifter sorted into size classes (0-74 g, 75-249 g, 250-849 g and >850 g) and weighed.

Table 6.1. Location and zone of seed potato lines from Simplot Gowers' Line trial in Tasmania during 2002, 2003 and 2004 growing seasons to assess relative performance of seed lines and incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX).

Year and grower	Location	Zone[1]
2003 season (No. seed lines)		
20	Upper Natone, Ridgley, Natone, Riana	1
7	Wilmot and Sheffield	2
9	Scottsdale, St Helens and Tullendeena	3
4	Tunnack and Mount Seymour	6
2004 season (No. seed lines)		
21	Upper Natone, Ridgley, Natone, and Riana	1
5	Wilmot and Sheffield	2
5	Scottsdale, St Helens and Tullendeena	3
1	Blessington	4
2	Tunnack, Mount Seymour	6
2005 season (No. seed lines)		
31	Ridgley, South Riana, Upper Natone, Natone, Riana, Nietta, Calder, Yolla and Preston	1
3	Sheffield, Wilmot and Lower Barrington	2
7	Scottsdale, Bridport Branxholm, Tullendeena and Winnaleah	3
2	Woodstock	7

[1] Seed growing geographical location in Tasmania shown in Figure 3.2 (Chapter 3, p. 103).

6.2.2 Virus testing

A leaflet sample was collected from 20 different potato plants within each plot, from the central 5 m of the two rows of each plot approximately two weeks prior to senescence of the crop. In 2002 and 2004 leaflets for virus testing were collected from three replicate plots, with 40 and 44 seed lines sampled, respectively. In 2003, 34 seed lines were sampled from either two replicate or one replicate plot. Leaflets were collected on 5 March 2003, 5 April 2004 and 7 March 2005. Samples were stored at 5°C for no more than 5 days prior to virus testing. Leaflets were individually virus tested for PVS and PVX by DAS-ELISA (Appendix II).

6.2.3 Yield analysis

Correlation analysis was used to determine the relationships between virus incidences within plots and yield of plots. Regression analysis was used to investigate any significant relationships in more detail.

6.2.4 Detection of late season infection

To determine if potential late season infection may not have been detected serologically at the end of each growing season, tubers were collected at harvest and a grow out test conducted. Fifteen seed potato lines were selected from post harvest tubers (2004 season) with low to moderate PVS incidence detected in foliage before senescence of the 2004 Simplot Growers' Line trail. Twenty tubers were randomly

collected from a composite sample of the tubers harvested from plots on 12 May 2005. Tubers were stored at 4°C for 5 months. Tubers were removed from cold storage and maintained at room temperature (approximately 15°C) for 4 days prior to planting. Tubers were planted on 18 October 2005 in commercial potting mix contained in clear plastic bags (30cmx20cm) and maintained under aphid proof greenhouse conditions for 8 weeks. Plastic bags ensured foliage contact did not occur between plants to avoid mechanical transmission of PVS or PVX between plants. One leaflet sample was collected on 18 December 2005 at 61 DAP from each of the 20 plants for each of the 15 seed lines assessed. Samples were virus tested for PVS and PVX by DAS-ELISA (Appendix II) on 19 December 2005.

6.3 Results

6.3.1 PVS and PVX incidence in plots

PVS was prevalent in seed line plots, occurring in 37/40 (92.5%), 32/34 (94.1%) and 43/43 (100%), with mean incidence of 41.7%, 61.2% and 50.2% for seed lines in 2002, 2003 2004, respectively, (Table 6.2).

PVX was less prevalent, occurring in 9/40 (22.5%), 3/34 (8.8%) and 11/43 (25.6%) seed lines with a mean incidence of 6.8%, 1.1% and 1.6% for 2002, 2003 and 2004 season, respectively (Table 6.2).

Table 6.2. Summary of incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) within Simplot Growers' Line trial seed lines in Tasmanian for 2002, 2003 and 2004 seasons.

	No. seed lines with virus	Average %	Minimum %	Maximum %	Standard deviation %
2002 season (total seed lines, 40)					
PVS	37	41.7	0	98.3	40.6
PVX	9	6.8	0	84.8	18.5
2003 season (total seed lines, 34)					
PVS	32	61.2	0	100	35.5
PVX	3	1.1	0	20	4.0
2004 season (total seed lines, 43)					
PVS	43	50.2	6.7	98.3	30.6
PVX	11	1.6	0	22.8	5.1

6.3.2 PVS and PVX incidence of seed sourced from different zones

2002 season

Frequency and occurrence of PVS and PVX during the 2002 season of seed lines sourced from geographical zones 1, 2, 3 and 6 in Tasmania are shown in Table 6.3. PVS were detected in 27.1% of the seed lines originating from zone 1 (Table 6.3). PVS was detected in 38.3% of seed lines from zone 2, (Table 6.3). A total of 92.8% PVS incidence was detected in seed lines in zone 3 (Table 6.3). Plots originating from zone 6 had 7.1% PVS incidence in seed lines (Table 6.3). Mean incidence of PVX in seed lines of zone 1, 2, 3 and 6 was 0%, 0.2%, 0.7% and 0.8%, respectively (Table 6.3).

2003 season

In the 2003 season seed lines originated from zone 1, 2, 3, 4 and 6 (Table 6.3). The mean incidence of PVS in seed lines from zone 1, 2, 3, 4 and 6 was 56.7%, 55.5%, 85.5%, 70.0% and 70.0%, respectively (Table 6.3). In the 2003 season, PVX was detected only in seed lines originating from zone 1 and 2, with a mean incidence of 2.1% and 1.5% respectively (Table 6.3).

2004 season

In 2004 tubers in seed lines originated from zones 1, 2, 3 and 7, with a mean incidence of 45.1%, 71.7%, 76.4% and 10.1%, respectively (Table 6.3). PVX was detected in zone 1 and zone 7 only with a mean incidence of 6.3% and 1.7%, respectively (Table 6.3). 5.4% above 50% infection (Table 6.5).

Table 6.3 Mean incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) within seed lines of Simplot Growers' Line trial in Tasmania for the 2002, 2003 and 2004 seasons showing regional zones differences.

	Regional Zone[1]					
	1	2	3	4	6	7
2002 season						
PVS						
Total seed lines tested	20	7	9	-	4	-
Mean incidence (%)	27.1	38.3	92.8	-	7.1	-
Standard Deviation (%)	35.5	36.9	9.1	-	8.1	-
PVX						
Total seed lines tested	20	7	9	-	4	-
Mean incidence (%)	0	0.2	0.7	-	0.8	-
Standard Deviation (%)	0	1.1	2.9	-	2.9	-
2003 season						
PVS						
Total seed lines tested	21	5	5	1	2	-
Mean incidence (%)	56.7	55.5	85.0	70.0	70.0	-
Standard Deviation (%)	41.1	29.4	24.6	28.3	43.6	-
PVX						
Total seed lines tested	21	5	5	1	2	-
Mean incidence (%)	2.1	1.5	0	0	0	-
Standard Deviation (%)	6.1	4.7	0	0	0	-
2004 season						
PVS						
Total seed lines tested	31	3	7	-	-	2
Mean incidence (%)	45.1	71.7	76.4	-	-	10.1
Standard Deviation (%)	27.7	35.4	19.6	-	-	6.3
PVX						
Total seed lines tested	31	3	7	-	-	2
Mean incidence (%)	6.3	0	0	-	-	1.7
Standard Deviation (%)	17.6	0	0	-	-	4.1

[1] Seed grower regions in Tasmania shown in Figure 3.2 (Chapter 3, p. 103).

6.3.3 Relationship between PVS and yield

A statistically significant negative correlation was reported in each of the three seasons between the incidence of PVS and i) processing yield (100-850 g range) (t/ha) and ii) with the weight of tubers (t/ha) in the 250-850 g size range (Table 6.4). In addition in 2002, there was a significant negative correlation between PVS incidence in plots and the yield of tubers in the >850 g category (Table 6.4).

Table 6.4. Correlation coefficient (*r*) and level of statistical significance (*P*=) between incidence of *Potato virus S* (PVS) and processing yield and yield of different size categories of cv. 'Russet Burbank' from Simplot Growers' Line trial in Tasmania for the 2002, 2003, 2004 seasons.

	r	*P*=
2002		
Processing yield (t/ha)	-0.355	0.001
Tubers in size ranges (t/ha)		
0-74 g	-0.092	ns
75-249 g	-0.078	ns
250-850 g	-0.274	0.01
>850 g	-0.206	0.05
2003		
Processing yield (t/ha)	-0.288	0.05
Tubers (kg/plot)		
0-100 g	-0.059	ns
101-249 g	0.074	ns
250-850 g	-0.325	0.05
>850 g	-0.216	ns
2004		
Processing yield (t/ha)	-0.365	0.001
Tubers in size ranges (t/ha)		
0-100 g	-0.021	ns
101-249 g	-0.090	ns
250-850 g	-0.299	0.001
>850 g	-0.126	ns

r = correlation coefficient, ns = correlation coefficient is not significant

In each of the three years there was a highly significant negative linear relationship between the incidence of PVS in plots and the processing yield (Figures 6.1, 6.2 and 6.3). In 2002 season the predicted yield at 0 and 100% PVS was 64.9 and 59.3 t/ha respectively, a difference of 5.6 t/ha. In 2003 season the predicted yield at 0 and 100%

PVS was 65.8 and 59.5 t/ha respectively, a difference of 6.3 t/ha. In 2004 season the predicted yield at 0 and 100% PVS was 72.6 and 62.5 t/ha respectively, a difference of 10.1 t/ha (Figures 6.1, 6.2 and 6.3). While the regression equation was highly statistically significant, it should be noted that there was considerable scatter of data points around the line leading to low R^2 values and reducing the predictive power of the analysis.

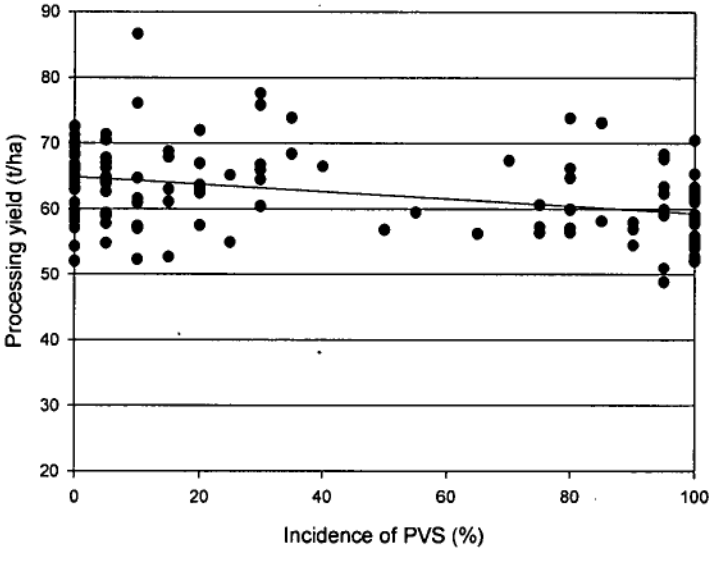


Figure 6.1. Regression between incidence of *Potato virus S* (PVS) and processing yield in Simplot Growers' Line trial during the 2002 season. Y=64.91 + -0.056X, $P<0.0001$, $R^2=0.08$.

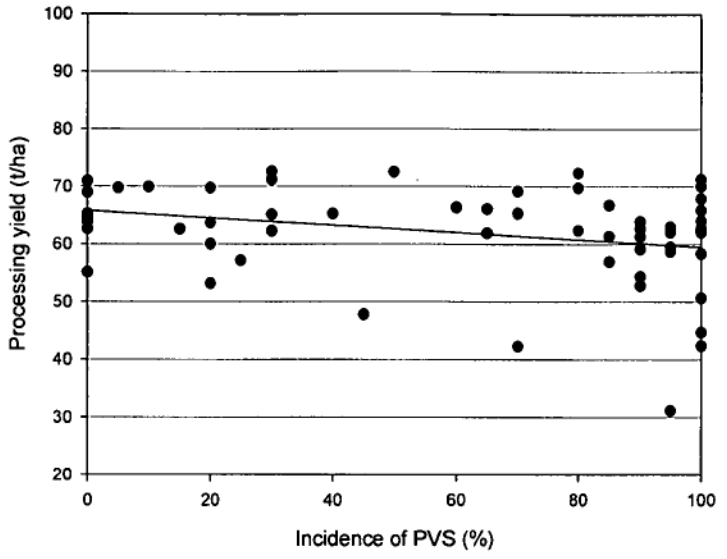


Figure 6.2. Regression between incidence of *Potato virus S* (PVS) and processing yield in Simplot Growers' Line trial during the 2003 season. Y=65.824 + -0.063X, P=0.03, R^2=0.08.

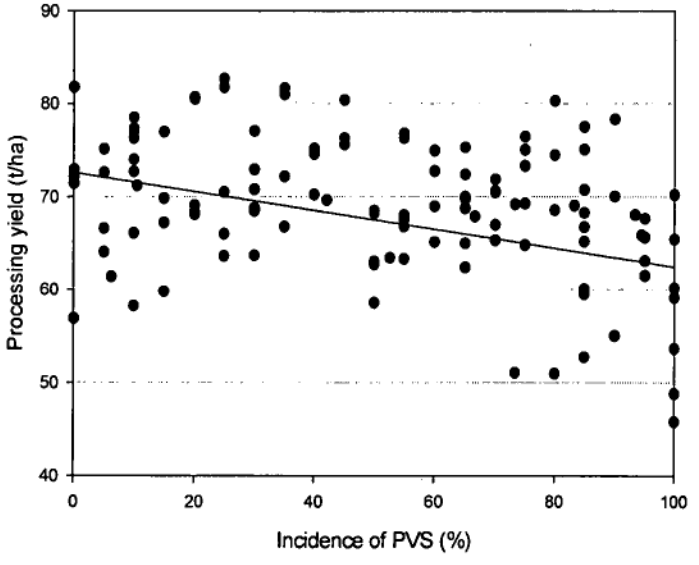


Figure 6.3. Regression between incidence of *Potato virus S* (PVS) and processing yield in Simplot Growers' Line trial during the 2004 season. Y=72.61+ -0.101X, P<0.001, R^2=0.16.

6.3.4 Effect on yield of mixed infection of PVS and PVX

Mixed infection of PVS and PVX have been reported to cause larger yield reductions than single infections with either virus alone (Stevenson *et al.* 2001). The data sets did not have sufficient combinations over a range of virus incidence to assess this hypothesis. However, in 2002 season four plots with high levels of PVX had average yield only slightly below those with no virus and similar yield to those that had high levels of PVS alone (Table 6.5). This suggests that combinations of PVS and PVX might not reduce yield any more than single infections of PVS alone. Data sets for 2003 and 2004 had insufficient combinations of high PVS and high PVX incidence for analysis.

Table 6.5. Processing yield (t/ha) selected plots from the 2002 season Simplot Growers' Line trial in Tasmania, containing different virus combinations of *Potato virus S* (PVS) and *Potato virus X* (PVX).

		Yield (t/ha)			
	No. of plots	Average	Minimum	Maximum	Standard deviation
No Virus	21	64.3	52.1	72.6	5.1
High PVS (>75%)	32	59.9	48.9	73.8	6.2
High PVX (>80%)	3	63.1	54.3	68.5	7.6
High PVS (>95%) & high PVX (45-75%)	4	60.6	58.4	63.4	2.3

6.3.5 Detection of late season infection

Virus incidence of PVS from post harvest foliage samples increased between 15-75%, compared to field samples for all seed lines tested (Table 6.6). PVX was detected in two seed lines for field samples and only one post harvest sample, with an increase of 5%. (Table 6.6). A paired two sample t-test was conducted with the null hypothesis of no significant difference between mean PVS incidence in field and in the grow out test. The t-statistic was −12.23 (df=14) significantly higher (P<0.0001) than t-critical (2.14) indicating a statistically significant mean difference.

Table 6.6. Summary of incidence *Potato virus S* (PVS) and *Potato virus X* (PVX) with Simplot Growers' Line trial plots in Tasmania, for 2004 season and virus incidence of foliage from tubers collected at harvest (2004).

			Field samples		Post harvest samples	
Line No.	**Origin (Zone)**	**Rep. No.**	**PVS %**	**PVX %**	**PVS %**	**PVX %**
2	Wilmot	1	5	0	80	0
3	Sheffield	1	30	0	63	0
5	Yolla	1	5	0	60	0
6	West Pine	1	35	0	85	0
13	Riana	1	65	0	100	0
21	Tewkesbury	1	5	0	75	0
28	Upper Natone	1	10	5	75	0
29	Natone	1	50	0	100	0
31	Riana	1	55	0	100	0
32	Riana	1	60	0	100	0
34	Scottsdale	1	85	0	100	0
37	Smithton	1	40	10	95	15
43	Wynyard	1	5	0	68	0
47	Woodstock	1	10	0	50	0
48	Woodstock	1	10	0	60	0

6.4 Discussion

This trial corroborates overseas studies that have suggested that PVS has a moderate effect on yield of potato. Correlation analysis showed a significant negative relationship between the incidence of PVS and processing yield (t/ha) of Russet Burbank in all three seasons. Regression analysis demonstrated that, on average, plots completely infected with PVS yielded 91.4%, 90.4% and 86.1% that of plots with no PVS, a difference of some 5.6 t/ha, 6.3 t/ha and 10.1 t/ha, in 2002, 2003 and 2004 season respectively. Negative relationships were also shown between the incidence of PVS and yield of tubers in the larger size ranges, suggesting that the effect on yield was, at least in part, through reduced tuber size. As many other factors in addition to virus are important determinants of yield, there was considerable variation around the regression line and low R^2 values. Results indicate that the magnitude of yield loss caused by PVS in Russet Burbank in Tasmania is of concern, and continued effort should be made to maintain low incidence of PVS in potato crops.

Overseas studies have shown PVS to cause yield losses of 0-15% (Stevenson *et al.* 2001). Limited work has been conducted on the effect of these viruses on potato yield in Australia. A preliminary investigation of the effect of generation and incidence of PVS on yield of potato in Tasmania (Mulcahy 2000), demonstrated no statistical difference between yield of cv. Russet Burbank G2 with no detectable virus and G15 with complete infection, with yields of 49.0-51.6 t/ha and 49.5-53.5 t/ha respectively. However in cv. Kennebec there was a significantly greater mean processing yield (17.2%) in G2 in comparison to G17 with complete infection with PVS, with mean yields of 69.5 t/ha and 57.6 t/ha, respectively (Mulcahy 2000).

Combinations of PVS and PVX have been reported to cause larger yield reductions than single infections with either virus alone (Stevenson *et al.* 2001). Insufficient combinations over a range of virus incidence in the data set did not allow for analyses of this. However, in 2002 season high levels of PVS and moderately high levels of PVX in four plots had an average yield only slightly below those with no virus and similar yield to those that had high levels of PVS alone. This suggested that combinations of PVS and PVX under trial conditions of the Simplot Growers' trial might not have reduced yield any more than single infections of PVS alone.

The limited data available for PVX suggested that the strain of PVX present in the trial may have minimal impact on yield of Russet Burbank when it occurred as single infections, and that combinations of PVS and PVX had no more impact than PVS alone. However, this situation could change if other more virulent strains of PVX entered Tasmania.

PVS incidence was higher in foliage from Growers' line tubers grown out the following season than in foliage tested late in the season, suggest primary infection was not detected. Virus incidence was 15-75% higher in foliage of post harvest tubers than in foliage tested late in the growing season of the Growers' line trial. Franc and Banttari (1983) report detection of PVS by ELISA in foliage between 13 and 20 days after mechanical inoculation of potato plants. de Bokx (1968) and de Bokx and Waterreus (1967) report translocation of PVS from inoculated leaves to daughter tubers occurred within 13 days for cvs. Russet Burbank and Norland, and within 14 days for European cultivars and 20 days for Kennebec. These findings suggest a late

season infection may have occurred and there was insufficient time for adequate replication to enable viral detection by DAS-ELISA.

Results of a survey in Tasmania during 2002/2003 demonstrated that PVS was present in 69% of 235 crops. The majority of crops (63%) had no PVS or incidences below 10%. Therefore on the basis of the Grower's Lines Trial, PVS is currently unlikely to be having a major impact on yield of Tasmanian crops. However, some 15% of crops had incidences of PVS above 50%, and these crops might be expected to be experiencing significant yield penalties. The widespread nature of PVS in seed crops is of concern as the virus is easily mechanically transmitted so there is potential for rapid increase up to levels at which yield losses become apparent. It is therefore important that steps be taken to reduce and/or eradicate PVS and PVX from Tasmanian seed potato.

7. Spatial patterns of PVS and PVX in Tasmanian seed potato

7.1 Introduction

The rate at which a plant disease epidemic progresses and the spatial pattern of the pathogen/disease in the field with time can provide valuable insight into the mechanisms of spread and inoculum source. Knowledge of the spatial distributions of pathogens can also provide essential information for sampling programs, experimental design and for simulation and modeling activities for epidemiology and disease management (Campbell and Madden 1990).

The absence of obvious symptoms of PVS infecting potato causes difficulties in identifying the presence of the virus and determining the spread of PVS in the field. As a result, limited studies have been conducted on the field spread of PVS with many studies pre-dating improved detection methods such as the serological technique of ELISA.

Infections of tubers or plants are considered to be either primary or secondary. Primary infection refers to healthy tubers or potato plants of the current season that become infected with virus within that season. Generally plants possessing primary infection tend not to elicit symptoms where inoculation has occurred after the initial formation of the tuber. However, symptoms resulting from infected plants are influenced by cultivar, inoculum level and environmental conditions. Secondary (chronic) infection results from using seed infected from the previous season and can

result in substantially reduced yield depending upon virus strain and cultivar used (Ragsdale *et al.* 2001).

Research in other countries has shown PVS and PVX to be spread predominately by mechanical transmission, e.g. during seed cutting operations (Franc and Banttari 1984; Larson 1950) and by contact between plants or from machinery movement within the crop (Beemster and de Bokx 1987; Brunt and Loebenstein 2001; Franc and Banttari 1984; Manzer and Merriam 1961). In addition, some strains of PVS are spread in a non-persistent manner by some aphid species (Wardrop *et al.* 1989). Aphid species identified as vectors of aphid transmissible isolates of PVS include *Aphis nasturtii* (buckthorn aphid), *A. fabae*, *Rhopalosiphum padi* L. (bird cherry/oat aphid) and *Myzus persicae* Silz. (green peach aphid) (Brunt and Loebenstein 2001). Of these, *R. padi* and *M. persicae* are present in Tasmania (L. Hill, DPIW, *personal communication*). However the range of species tested as vectors of PVS is limited and there may be other, as yet unknown, vector species. For example, *Capitophorus eleaeagni* was recently identified as a potentially important aphid vector of PVY in Idaho, U.S.A. (Halbert *et al.* 2003).

Weed hosts could also serve as a source of inoculum for PVS and PVX. The host range of PVS includes *Chenopodium quinoa* L. and *C. amaranticolor* L. (Brunt and Loebenstein 2001), while PVX has a limited host range constrained to solanaceous species such as *Solanum nigrum* L., *Nicotiana* spp., *Petunia hybrida* L., *Datura stramonium* L., *Cyphomandra betacea* (Cav.) Sendt. and *Lycopersicon esculentum* Mill. (Brunt and Loebenstein 2001). Volunteer potato from previous crops may also act as an effective reservoir of inoculum (Wright and Bishop 1981).

Plant disease epidemiology refers to the comprehensive analysis of an epidemic, resulting from the study of interactions between factors of the disease triangle - host, pathogen and environment (Jones 1998). An epidemic results when disease intensity increases over space and time within a host population (Campbell and Madden 1990). Valuable information can be gained from studying spatial patterns of epidemics including inoculum information (primary or secondary inoculum), dispersal mechanisms of pathogen, and influence of environmental conditions upon the epidemic. Important epidemiological information can be obtained by studying spatial patterns of infected plants within the field. Diseased plants that are of an aggregated (cluster) pattern suggest that pathogen spread is occurring from plant to plant within a field. Diseased plants displaying a random pattern indicate pathogen spread is not occurring from plant to plant, at observation time (Madden *et al.* 1982). The study of plant disease epidemics such as those caused by viruses, provides important information necessary to develop cost effective management strategies to minimise the economic impact of disease in crops. Some of the more commonly used spatial analysis techniques for plant pathology are listed below with emphasis on techniques such ordinary runs and spatial analysis by distance indices.

Campbell and Madden (1990) discussed methodologies for spatial analyses of plant diseases based upon ways in which plants are sampled, i) position within a row (or series of rows) of diseased or healthy plants, ii) count data from a plot or quadrat, and iii) distance measurements. Other considerations to take into account when deciding which category to choose are circumstances that are beyond the control of the researcher (e.g. the inability to measure distance between individual fungal propagules

in the soil) (Campbell and Madden 1990). Two spatial analysis techniques commonly used in plant disease epidemiology were used in this study and are described below.

Ordinary runs analysis

Ordinary runs analysis can be used to determining aggregation of infected plants Madden *et al.* (1982). A run refers to one or more healthy or diseased plants occurring in succession. Expected number of runs with a null hypothesis of randomness is give by Equation 7.1.

$$E(U) = 1 + \frac{2m(N-m)}{N}$$

Equation 7.1

Infected plants are considered clustered when the expected number $[E(U)]$ of runs is more than the observed number of runs (Gibbons 1976; Campbell and Madden 1990). Under the null hypothesis the standard deviation of U is given by Equation 7.2.

$$s(U) = \left(\frac{2m(N-m)[\,2m(N-m) - N]}{N^2(N-1)} \right)^{1/2}$$

Equation 7.2

Clustering is determined by the Z-statistic, where $Z = [U - E(U)]/s(U)$, Z values less than -1.64 (P=0.05) suggest aggregation of infected plants. It should be noted where N is less than 20 this test has reduced power to detect randomness (Campbell and Madden 1990). The units are also required to be at regular spacings.

Spatial Analysis of Distance IndicEs (SADIE)

Perry and Hewitt (1991) described a new method, SADIE. Information of the relative position of sampling units is required, to enable mapping onto a Cartesian plane. Essentially SADIE measures the total distance (D) (effort expended) individuals are required to move to achieve an extreme arrangement, where individuals of the sample are uniformly or regularly spaced as possible so each sampling unit consists of equal numbers of diseased individuals. Comparisons can then be made between observed D and distance to regularity. I_a (index of aggregation) is a SADIE statistic given by D/E_a, where E_a "is the arithmetic mean distance to regularity for randomised samples" (Xu and Madden 2004). Where, $I_a > 1$ indicates an aggregated spatial pattern of diseased plants, $I_a = 1$ suggests a random pattern of diseased plants and $I_a < 1$ indicates a regular spatial pattern (Perry 1998). I_a is commonly used to detect nonrandomness in spatial pattern studies in plant pathology (Pethybridge and Turechek 2003; Pethybridge *et al.* 2005; Scott *et al.* 2003).

Later the expansion of the SADIE technique enabled graphical display of spatial data (Perry *et al.* 1999; Xu and Madden 2004). Clustering indices (described below) can be used to provide a graphical two-colour (red-blue plots) display of cluster detection and measure for spatial patterns of count data (Perry *et al.* 1999). Cluster indices are calculated from the net distance of individuals from sampling units to randomisation, include v_i (greater observed counts than the mean, positive value) and v_j (fewer observed counts than the mean, negative value) (Xu and Madden 2005). Areas with v_i greater than the 95th percentile of v_I (from randomisations) are considered patch clusters. Areas with v_j less than the 95th percentile of v_j (from randomisations) are referred to as gap clusters.

The objectives of this study were to provide information on the epidemiology of PVS and PVX in Tasmania by: i) quantifying spatial patterns of PVS and PVX infected plants during crop development; ii) characterising the temporal progression of PVS and PVX epidemics; iii) quantifying the spatiotemporal relationships between infected plants at successive time periods; iv) assessing the role of weeds as alternative hosts for PVS and PVX, and v) determining the role of aphids in transmission of PVS. Information from this study will assist the Tasmanian potato industry in the development of cost-effective strategies for control of these viruses.

7.2 Materials and methods

7.2.1 Field sites and data collection for spatial analyses

Two trials ('plots' and 'transects') were established in each of four commercial fields of seed potato cv. 'Russet Burbank' over two years. Plot trials consisted of 49 plots, arranged in a seven by seven lattice. Twenty leaflets were collected at random from each plot at each sampling time and tested for virus (below). Transect trials consisted of seven adjacent rows, each 50 m long. At each sampling time, one leaflet was collected at 1 metre intervals along each row and tested for virus (below).

In 2003, two fields (fields 1 and 2) were located at Riana (UTM coordinate: 55G 414798 5434841) in north west Tasmania. Field 1 (G4) was planted on 10 December 2003 and plots were 10 rows wide (8 m) x 10 m long, with approximately 500 plants/plot. Field 2 (G4) was planted on 20 November and plots were nine rows wide (8.3 m) x 10 m long, with approximately 450 plants per plot. Transect trials were established in each field as described above. Leaflets for virus testing were collected from field 1 at 58 and 129-130 days after planting (DAP) and from field 2 at 56-58 and 132-133 DAP. In 2004, one field (field 3) was located at Riana and the other (field 4) at Scottsdale (UTM coordinate 55G 541955 5444470) in north east Tasmania. In field 3 the plots (G3) and transects (G4) were planted on 19 November and field 4 (G3) was planted on 17 November. Plots within field 3 consisted of eight rows wide x 10 m long (8.3 m wide x 10 m long), with approximately 480 plants per plot. Plots within field 4 consisted of seven rows wide x 10 m long (7 m wide x 10 m long), with approximately 420 plants per plot. Transect trials were established in each

field as described above. Leaflets for virus testing were collected from field 3 at 31 and 107 DAP and from field 4 at 30, 54 and 105 DAP. Commercial recommendations were followed for planting, fertiliser application and weed control at all fields. Irrigation water was applied by travelling gun irrigator at field 1 and 4 and by solid set sprinklers at fields 2 and 3.

7.2.2 Virus testing and virus incidence

Leaflets were assessed for the presence of PVS and PVX by DAS-ELISA (Appendix II). The Score interval method (Vollset 1993) was used to calculate lower and upper 95% binomial confidence limits (P=0.05) on the mean virus incidence at each assessment time.

7.2.3 Spatial and spatiotemoral analysis

Spatial patterns were depicted using geostatistical-based techniques. In transect trials spatial analysis was conducted by ordinary runs analysis (Madden *et al.* 1982) and on virus incidence in plots by Spatial Analysis by Distance IndicEs (SADIE Version 1.22) (Perry *et al.* 1996). Analysis was conducted only when virus incidence was greater than 5% and less than 95%.

For ordinary runs analysis a 'run' was defined as a succession of one or more like events (i.e. infected or uninfected plants). A non random distribution of infected plants was conducted (P = 0.05) if the Z-statistic calculated according to Madden *et*

al. (1982), was less than –1.64. Runs were assessed by joining plants both along annd across rows, to test for aggregation in both directions.

SADIE has been described previously (Perry 1995; Perry 1998; Perry *et al.* 1996; Perry *et al.* 1999; Xu and Madden 2004) (see Section 7.1). Briefly, SADIE uses a transportation algorithm, to calculate the shortest distances required to move spatially referenced data to obtain both 'regular' and 'crowded' spatial patterns. The overall distances required for these moves are then summed and compared to random simulations based on re-sampling of the diseased measure locations. All simulations used the maximum number of randomizations (n = 5967). Deviation of the index of aggregation (I_a), the ratio of the expected and observed distances to regularity, from the null hypothesis of no spatial dependence was assessed by a one-sided test for aggregation. Values of I_a equal to one indicate a random spatial pattern, values less than one indicate a regular pattern and values greater than one, an aggregated pattern.

Temporal associations in spatial patterns between two consecutive sampling times were analysed using the Association Extension of SADIE (Version 1.22) (Winder *et al.* 2001). Overall association (X) was calculated as the mean of individual local associations between the clustering indices, which estimate the net distance individuals are required to move to achieve regularity. Significance of X was tested by the maximum number of randomisations of the local association values, allowing for small-scale autocorrelation with the Dutilleul adjustment, using a two-tailed test. The null hypothesis of no association was used (Winder *et al.* 2001).

Cluster indices generated from SADIE were used to provide a graphical display of a two-colour ('red-blue plots') presentation of cluster detection and measure for spatial patterns of count data (Perry *et al.* 1999). SigmaPlot software (version 7.0, SPSS Inc., Chicago, U.S.A.) was used to plot cluster indices onto contour plots to produce maps of gap and patch clusters.

7.2.4 Prevalence and incidence of PVS and PVX in weeds

The prevalence and incidence of both viruses in weeds were assessed at each field. Leaf samples were collected from weeds arbitrarily within plots and at edges of each field were tested for PVS and PVX by DAS-ELISA (Appendix II). Leaves were collected from fields 1, 3 and 4 at 130, 107 and 105 DAP, respectively and from field 2 at 77 and 133 DAP.

7.2.5 Aphids

To monitor flights of alatae (winged) aphid, two yellow sticky aphid traps (9.5 cm wide x 23.0 cm long) were placed 1 metre above ground level at the field edge facing into the prevailing wind. Traps were placed in fields 1-4 at 80, 70, 31 and 30 DAP, respectively, and replaced at weekly (fields 1 and 2) or fortnightly (fields 3 and 4). Trapping occurred between 3 February 2004 and 7 April 2004 (fields 1 and 2), 20 December 2004 and 7 March 2005 (field 3), and 7 December 2004 and 3 March 2005 (field 4). Traps were stored at 10°C until processed for aphid identification by C. Young, DPIW. Aphids were located under a dissecting microscope (50 X) and removed from traps by soaking in a dipentine-based solvent (DeSolvit™, RCR International, Victoria, Australia). Aphids were identified to species using keys

(Blackman and Eastop 2000) and by comparison with type specimens held in the insect collection at the DPIW, New Town, Tasmania.

7.2.6 Virus testing of stunted plants

During the 2003 growing season 20 leaflet samples were collected at field 2 from plants exhibiting symptoms of stunting comparative to adjacent plants and were tested for PVS and PVX by DAS-ELISA (Appendix II). Ten leaflets from an additional 10 plants considered to be healthy were also collected from field 2 and tested for PVS and PVX by DAS-ELISA (Appendix II). Samples were collected on 3 February 2004 at 76 DAP.

7.2.7 Weather monitoring

Temperature and rainfall was monitored during the season with a datalogger (Watchdog 450, Spectrum Technologies) at two field between 6 January 2004 - 14 March 2004 (field 1) and 14 January 2004 – 28 March 2003 (field 2) (Appendix IV).

7.2.8 Post harvest virus testing of tubers

Three hundred tubers from field 1 and field 2, and 100 tubers from field 3 were collected arbitrarily after harvest and virus tested to compare virus incidence in tubers with that of plants during the season. Tubers were collected from one half-tonne bin following commercial harvest of the field. Tubers were collected from field 1, field 2 and field 3 at harvest on 10 April 2004, 15 March 2004 and 8 March 2005, respectively. Tubers were stored at 4°C for 5 months. Tubers from field 1 and field 2

were placed in individual containers and maintained at room temperature (approximately 15°C) for 6 weeks, to promote sprout formation. At 6 weeks a 1cm section of one sprout (approximately 5-25 cm long) from each tuber was collected and virus tested for PVS and PVX by DAS-ELISA (Appendix II). Tubers collected from field 3 were stored for 3 months at 4°C. Tuber dormancy was broken by placing tubers at room temperature (15°C) for 4 days. Sprouts (0.5 cm) were collected on 24 August 2005 (6 weeks after being placed in individual containers) and virus tested for PVS and PVX by DAS-ELISA (Appendix II). Tubers were planted on 24 September 2005 in commercial potting mix and grown in aphid proof cages in a greenhouse. A leaflet from each plant was collected on 28 November 2005 (65 DAP) and tested for PVS and PVX by DAS-ELISA (Appendix II).

7.3. Results

7.3.1 Virus incidence

PVS was present in plots and transects in all fields (Table 7.1). No change was detected in the incidence of PVS in plots or transects of fields 1 or 2, with the 95% CI's around the mean incidence overlapping at each sample time (Table 7.1). Significant spread of PVS was detected in plots (but not transects) of field 3, with an increase in incidence of 5.2% between 31 and 107 DAP (Table 7.1). In field 4 significant spread of PVS was detected in transects (but not plots) with an increase of 25.2% between 54 and 105 DAP (Table 7.1).

PVX was detected in plots and transects of field 2 and 3 (Table 7.1). PVX was not detected in plots and transects of field 1 early in the season. Later in the season a trace amount (0.1%) of PVX was detected in plots of field 1, but not in transects (Table 7.1). Similarly in plots and transects of field 4, no PVX was detected throughout the season (Table 7.1). In field 2, no increase in PVX was detected in plots or transects. A significant increase in the incidence of PVX occurred in transects (but not plots) of field 3, with an increase of 10.1% between 31 and 107 DAP (Table 7.1).

Table 7.1. Mean incidence (and 95% confidence intervals) of *Potato virus S* (PVS) and *Potato virus X* (PVX) in cv. Russet Burbank seed potato in plot[1] and transect[2] trials from four seed potato fields in Tasmania during 2003 and 2004.

Year, location and days after planting (DAP)	Mean PVS incidence (%)	Mean PVX incidence (%)
Field 1		
Plots		
58	50.2 (40.1-53.3)[3]	0 (0.0-0.4)
129	45.3 (42.2-48.4)	0.1 (0.0-0.6)
Transects		
58	52.9 (47.8-58.1)	0 (0.0-1.0)
130	57.1 (52.0-62.2)	0 (0.0-1.0)
Field 2		
Plots		
56	0.5 (0.2-1.2)	5.4 (4.2-7.0)
132	0.6 (0.3-1.3)	7.7 (6.2-9.5)
Transects		
58	0.3 (0.1-1.6)	6.2 (4.1-9.2)
133	1.1 (0.0-2.9)	8.4 (6.0-11.7)
Field 3		
Plots		
31	10.8 (9.0-12.9)	2.8 (1.9-4.0)
107	16.0 (13.9-18.5)	4.1 (3.0-5.5)
Transects		
31	15.4 (12.0-19.5)	26.3 (22.0-31.1)
107	17.9 (14.3-22.2)	36.4 (31.6-41.5)
Field 4		
Plots		
30	26.3 (23.7-29.2)	0 (0.0-0.4)
54	21.1 (18.7-23.8)	0 (0.0-0.4)
105	26.2 (23.6-29.1)	0 (0.0-0.4)
Transects		
30	55.2 (50.0-60.3)	0 (0.0-1.1)
54	55.5 (50.0-60.3)	0 (0.0-1.1)
105	80.7 (77.8-85.7)	0 (0.0-1.1)

[1] 980 leaflets
[2] 357 leaflets tested at each time interval
[3] 95% confidence intervals around mean virus incidence calculated by the Score method (Vollset 1993)

The distribution of PVS and PVX infected plants in transect trials from field 1, 2, 3 and 4 are shown in Appendix IV. A visual representation of PVS and PVX incidence in plots at field 1 (58 and 129 DAP), field 2 (58 and 129 DAP), field 3 (31 and 107 DAP) and field 4 (30, 54 and 105 DAP) are shown in Appendix V.

7.3.2. Spatial Analysis

Spatial analysis of PVS incidence by SADIE detected a random spatial pattern of infected plants in plots in fields 1 and 3 and an aggregated spatial pattern at all times in field 4 (Table 7.2). Ordinary runs analysis of transects demonstrated aggregation of PVS infected plants along (but not across) rows at early and late season in field 1, and aggregation across (but not along) rows at early season in field 3 (Table 7.2). In field 4, ordinary runs analysis detected aggregation along rows at early, mid and late season and across rows at early and mid-season. The incidence of PVX was sufficient for analysis (>5% incidence) by SADIE only in plots of field 2 and by ordinary runs analysis in transects of field 2 and 3. A random distribution of PVX infected plants was detected by SADIE in plots of field 2 and along and across rows in transects by ordinary runs analysis in fields 2 and 3 at all times (Table 7.2).

Table 7.2. Spatial analysis of the incidence of *Potato virus S* (PVS) and *Potato virus X* (PVX) in seed potato from four fields in Tasmania at intervals during 2003 and 2004 season using Spatial Analysis by Distance IndicEs (SADIE) and ordinary runs analyses.

Field	Days after planting	SADIE (plots) I_a[1]	Ordinary runs (transects)[2]	
			Across rows	Along rows
Field 1 (PVS)	56	0.80 ns[3]	1.02 ns	-1.96*
	129	1.81 ns	-1.23 ns	-2.96*
Field 2 (PVX)	56-58	1.22 ns	1.49 ns	0.56 ns
	132-133	1.21 ns	-0.51 ns	-0.51 ns
Field 3 (PVS)	31	0.98 ns	-2.56*	-1.33 ns
	107	0.90 ns	0.26 ns	0.26 ns
Field 3 (PVX)	31	-[4]	0.55 ns	0.0002 ns
	107	-	0.36 ns	1.74 ns
Field 4 (PVS)	30	1.61**	-2.05*	-3.12*
	54	1.36*	-1.94*	-2.58*
	105	1.64**	-1.00 ns	-6.06*

[1] I_a is the index of aggregation.

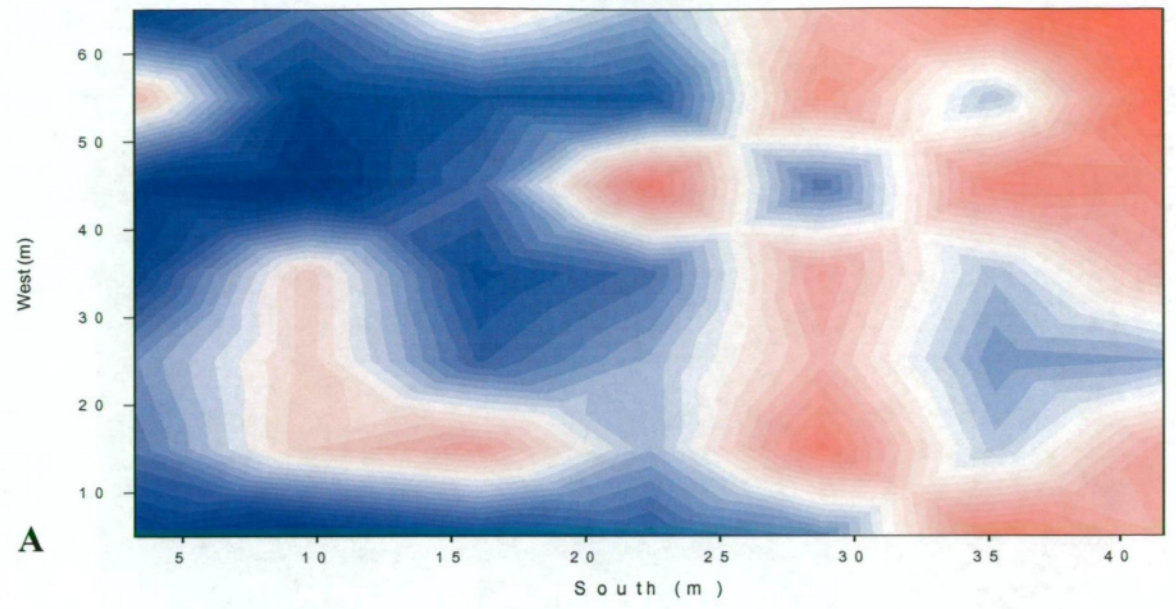
[2] Z statistics in two dimensions (across and along rows)

[3] ns (not significant), * ($P \leq 0.05$), ** ($P \leq 0.01$)

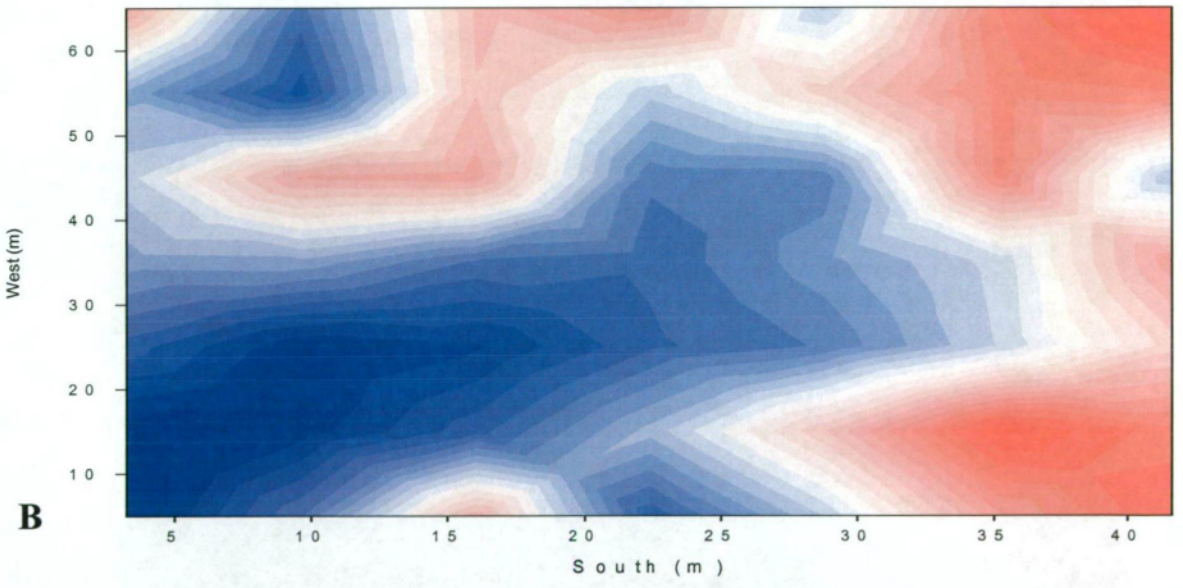
[4] Not analysed due to virus incidence being too low (<5%) for spatial analysis.

Significant spatial association was detected between the distribution of PVS infected plants at 30 and 56 DAP (X=0.376; P=0.006) and 56 and 105 DAP (X=0.384; P=0.007) in field 4. However, no significant spatial association was detected in spatial distribution of virus infected plants in plots between time periods at other fields (data not shown).

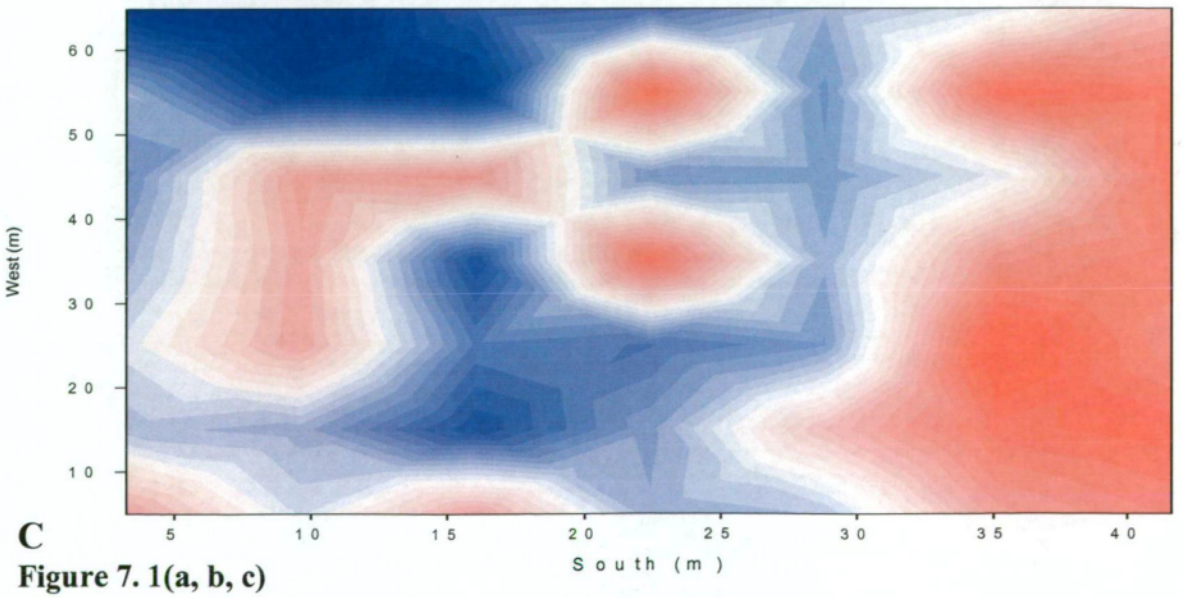
Clustering indices generated by SADIE for each time period where spatial aggregation was determined (in field 4 only) provided spatial position of disease foci (Figure 7.1). Areas of significantly high incidence (patch clusters) were situated at the eastern end of the plot trial at 30, 54 and 105 DAP, and to lesser extent in the centre of the trial (Figure 7.1). For all times periods the western end of the trial was denoted by areas of significantly low PVS incidence areas (gap clusters) (Figure 7.1).



A



B



C

Figure 7. 1(a, b, c)

Key to Figure 7.1.(a,b,c)

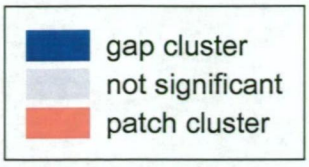


Figure 7.1. (a,b,c). Contour plots of clustering indices generated by Spatial Analysis by Distance IndicEs (SADIE) of *Potato virus S* (PVS) in cv. Russet Burbank seed potato field (field 4) in Tasmania, a) at 30 days after planting (DAP), b) 54 DAP, c) 105 DAP.

7.3.3. Prevalence and incidence of PVS and PVX in weeds

In field 1, PVS was not detected in samples of *Chenopodium album* L. (total number of samples, *n*=32) or *Solanum nigrum* L. (*n*=13). In field 2, PVS was not detected in samples of *C. album* (*n*=86), *S. nigrum* (*n*=37), *Polygonum persicaria* L. (*n*=28), *Rumex acetosella* L. (*n*=9), *Sisymbrium officinale* (L.) Scop. (*n*=31), *Fagopyron esculentum* Moench. (*n*=2), *Trifolium repens* L. (n=5), *Geranium dissectum* L. (*n*=1), *Carduus tenuiflorus* (Curt.) (*n*=1), *Coronopus didymus* (L.) Sm. (*n*=1), *Senecio vulgaris* L. (*n*=2), *Sonchus asper* (L.) Hill (*n*=2), *Rumex crispus* L. (*n*=1), and *Capsella bursa-pastoris* (L.) Medik. (*n*=3). PVS was detected in 1 of 2 *C. album* and 2 of 58 *S. nigrum* collected in field 3. In field 4, PVS was detected in 2 of 53 *C. album*, 1 of 11 *Malva sylvestris* L. and 1 of 6 *Rumex obtusifolius* L. In addition, PVS was not detected in samples of *S. nigrum* (*n*=37), *Raphanus raphanistrum* L. (*n*=1), or *Amaranthus powellii* S. Wats. (*n*=1) in field 4. PVX was not detected in weed samples collected from any of the fields.

7.3.4 Aphids

Small numbers of aphids were trapped, mostly late in the development of the potato crops. In field 1, *Aphis gossypii* Glover (melon/cotton aphid) occurred on traps retrieved on 10 March (n=1), 1 April (n=1) and 7 April 2004 (n=2) and *Macrosiphum euphorbiae* Thomas (potato aphid) occurred on traps retrieved on 7 April 2004 (n=1). In field 2, *A. gossypii* occurred on traps retrieved on 17 March (n=2) and 7 April 2004 (n=28) respectively. No aphids were detected on traps in field 3. In field 4, a single *M. euphorbiae* was trapped on 3 March 2005.

7.3.5 Examination of stunted plants

PVS was not detected in any of the 20 stunted or 10 healthy plants collected from field 2. PVX was detected in stunted plants (10%) and in healthy plants (5%), indicating no apparent relationship between these viruses and stunting at this site.

7.3.6 Post harvest virus infection

Virus incidence of PVS from tubers collected from across field 1 at harvest was 71% (Table 5.3), in comparison to virus incidence in foliage from both plots and transects at 130 DAP of 25.7% and 13.9%, respectively. PVX was not detected in tubers collected from field 1, although trace infection (0.1%) was detected in foliage in plots (but not transects) at 129 DAP.

Table 7.3. Incidence (%) of *Potato virus S* (PVS) and *Potato virus X* (PVX) in tubers collected from three field trials from seed potato fields in Tasmania during 2003 and 2004 seasons.

	Number of samples tested	PVS %	PVX %
Field 1	300 sprouts[1]	71.0 (65.6-75.8)[4]	0 (0-1.3)
Field 2	300 sprouts[1]	10.7 (7.7-14.7)	16.3 (12.5-20.9)
Field 3	100 sprouts[2]	32.0 (23.7-41.7)	80.0 (71.1-86.7)
Field 3	100 foliage[3]	38.0 (29.1-47.8)	81.0 (72.2-87.5)

[1] Sprouts tested by DAS-ELISA at 6 weeks after cool storage of tubers

[2] Sprouts tested by DAS-ELISA at 6 weeks after cool storage of tubers

[3] Foliage tested at 8 weeks after planting tubers in aphid proof house

[4] 95% confidence intervals around mean virus incidence calculated by the score method (Vollset 1993)

In field 2 PVS incidence in tubers collected post harvest was 10.7% (Table 7.3), which compared with an incidence in foliage from plots and transects at 132-133 DAP of 0.5 and 0.6%, respectively (Table 7.1). An increase was reported for PVS and PVX in both plots and transects. The incidence of PVX in tubers from field 2 was 16.3% (Table 7.3) which compared with that in foliage of plots and transects at 132-133 DAP of 7.7 and 8.4 % respectively (Table 7.1).

In field 3 virus incidence of PVS and PVX in tubers based on testing sprouts at 6 weeks after cool storage (4°C for 5 months) was 32% and 80%, respectively. Virus incidence of PVS and PVX of plants grown from these tubers at 8 weeks after planting was 38% and 81%, respectively. This compared with an incidence in foliage

at 107 DAP of PVS of 16.0% (plots) and 17.9% (transects) and of PVX of 4.1% (plots) and 36.4% (transects).

7.4 Discussion

Limited increase in incidence of PVS and PVX was detected in Tasmanian seed potato crops during the growing seasons. An increase in the incidence of PVS was detected in only one of the four plot trials (5.2% at field 3) and one of the four transect trials (25.5% at field 4). Similarly no significant increase in PVX incidence was detected in any of the three plot trials where PVX was detected and only one of the two transect trials (10.1% at field 3). In those trials in which PVX was not detected early in the season, either none or only trace amounts of PVX were detected late in the season, suggesting no transmission from external sources.

Spatial analyses of PVS incidence in plots in fields 1 and 3 by SADIE suggested a random distribution throughout the season. This distribution was indicative of the planting of infected tubers, with no evidence of further spread during the season. However, aggregation along rows was detected by ordinary runs analysis in field 1, suggesting some mechanical transmission of PVS between tubers at planting, as no further increase of PVS was detected during the season. In field 4, SADIE detected aggregation at all times and ordinary runs analysis detected aggregation along and across rows at early and mid season, but only along rows at late season. Aggregation of infected tubers at planting may result from the planting of seed from particular bins that had been harvested from areas of high virus incidence the previous season, or virus transmission between tubers within particular bins by seed cutting prior to planting or by mechanical transmission during handling and planting of tubers. Sprouts can contain high virus concentrations (Franc and Banttari 1984) and may be damaged during planting operations, increasing the potential for transmission between

tubers at this time. The aggregation of PVS infected plants in transects of field 4 along, but not across rows, later in the season coincided with an increase in virus incidence and suggested mechanical transmission predominately along rows. This may reflect the closer spacing of plants along rows compared to across rows, leading to plants contacting each other along rows earlier in the season and therefore providing more opportunity for mechanical transmission in comparison to plants across rows.

The increase of PVS in transects, but not in plots in field 4 may have resulted from the former having a higher virus incidence and therefore increased opportunity for transmission between plants than the latter. In addition, transects were located at the field edge, while plots were mostly within the potato field. No traffic occurred along the field edge bordering transects to facilitate virus transmission. However, transects at the field edge may have been more exposed to wind damage than plots, with consequently more opportunity for mechanical transmission. Although aphids can preferentially alight at field edges, leading to a higher virus incidence at edges (DiFonzo *et. al.* 1996b), aphid trapping did not suggest the presence of known aphid vectors at any of the fields.

For PVX, random distributions were detected at all times in field 2 by both spatial analyses methods and in field 3 by ordinary runs analysis. As PVX is transmitted only by mechanical means (Brunt and Loebenstein 2001), any increase in incidence might have been expected to occur between adjacent plants, forming an aggregated distribution. Alternative means of transmission of PVX have been documented, such

as by chewing insects (Bercks 1970) or aerosols (Banttari and Venette 1980), which might account for the random distribution following spread in transects at field 3.

Limited increase in the incidence of PVS and PVX in Tasmanian fields is unlike the = findings of many overseas studies with increase virus incidence in subsequent generation (Cockerham 1958; Fletcher 1984; Fletcher *et al.* 1996; Franc and Banttari 2001; Kamenikova 1978). However, direct comparison with these studies is difficult as the majority of studies involved assessment of inter-generational reinfection rates, making it difficult to separate in-field spread with spread that may have occurred during seed cutting, storage or planting. In addition virus testing methods have improved greatly since some studies were conducted, and agronomic methods have changed considerably and differ between countries. In older trials, there were often considerable mechanical operations through the crop for weed control, moulding of rows, application of pesticides by ground equipment and the use of planting equipment with metal spikes to plant seed pieces. These agronomic operations may have led to significant mechanical virus transmission. By contrast, in the four fields studied in Tasmania, minimal traffic occurred through the crops with no moulding of rows, the use of aerial spraying, the use of irrigation via solid set sprinklers or travelling gun-type irrigators with wide laneways and the use of cup-type planters. These agronomic operations may have minimised mechanical virus transmission. In some trials, viruliferous aphids have been implicated as significant cause of reinfection of seed potato with PVS (Kamenikova 1978), while in others mechanical transmission has been considered more important (Fletcher 1984). In these trials, no known aphid vectors of PVS were detected on traps, and the minimal spread of PVS in all but field 4, suggested aphids did not contribute to transmission.

Although limited studies have been conducted, weeds have not been found to be important sources of PVS (Khalil and Shalla 1982; Thomas and Richards 2003). Serological testing in this study indicated PVS to occur infrequently in some weeds in fields studied. Several weeds have been shown to be sources of PVX in overseas studies (Allen and Davis 1982; Locatelli *et al.* 1978), however PVX was not detected in weeds in my study. These results suggest that weeds are unlikely to be a major source of inoculum in the particular fields studied. Further evidence for limited external sources of PVS inoculum is gained from the significant spatiotemporal association of PVS infected plants between time periods.

Survival of volunteer potato is a problem for the Tasmanian potato industry. The maritime climate of NW Tasmania and other potato growing regions of Tasmania provide ideal climatic conditions for the survival of volunteer potatoes that are problematic as a potential inoculum source of potato diseases (Mulcahy 2000). Additions to the National Standard for Certification of Seed Potato have been adopted by the Tasmanian seed potato industry (Tasmanian Standard, as set by DPIW). These amendments include a requirement of seed crops to be planted on land freed from potato for a minimum of five years for all generation. Comparatively the National Standard has a minium three-year rotation for G4 and G5 potato crops. Long potato rotations assist in minimising the impact of diseases carried by volunteer potatoes (Mulcahy 2000).

Virus incidence was higher in tubers collected from field trials than in foliage tested late in the season. There was 13.9-25.0%, 9.6-10.1% and 20.1-22.0% higher incidence

detected in tubers compared to foliage collected late season from plots and transects. Similarly there was a 7.4-8.6% and 44.6-76.8% higher incidence of PVX in tubers in comparison to that detected in foliage collected late season from plots and transects of fields 2 and 3, respectively. Franc and Banttari (1996) demonstrated detection of PVS by ELISA in foliage of greenhouse-grown potato between 13 and 20 after mechanical inoculation. Higher incidence of virus in tubers compared to leaf samples collected during the growing season may indicate a late season infection, which may not have been detected from leaf samples during the growing season. However, caution is required when interpreting these results as the location of tubers in the field at collection is unknown and may indicate an initially high virus incidence of these areas of the field rather than an increase late in the season that remains undetected in the field. However an increase in incidence in tubers compared with foliage was noted for PVS and PVX in 3/3 and 2/2 fields, respectively. A study to compare virus incidence in field leaf samples compared to Growers' Line trial (Chapter 6) found an increase of between 15-75% reported for PVS from post harvest foliage compared to foliage samples from fields of 15 seed lines. However, viral detection in field leaf samples may have had lower incidence of PVS compared post harvest foliage due to the inability of DAS-ELISA to detect a late season infection.

The increase of both PVS and PVX indicates mechanical transmission may occur late in the season prior to harvest. The presumption of mechanical transmission may be attributed to the lack of aphids trapped at each site reducing possible aphid transmission of PVS, and that PVX is only mechanically transmitted. Differences in detection between leaf samples during the growing season and sprouts of tubers may also depend on the nature of transmission. For PVS, aphids potentially inoculate

relatively small amounts of virus in comparison to mechanical inoculation and the former may take longer to attain detectable concentrations within the plant.

Mature plant resistance in potato provides a further complicating factor which can affect transmission rate of virus between plants and tubers. Therefore late season infections may not actually contribute to an increase in virus in the next generation. de Bokx (1968) reported mature plant resistance to PVS in European cultivars at four weeks after planting, which became more pronounced at six weeks. The degree of resistance varied with cultivar and with PVS isolate. However, Franc and Banttari (1996) were unable to detect mature plant resistance to a Minnesota isolate of PVS and postulated that this at least partially explained the rapid reinfection of healthy potatoes in Minnesota. Spread of European isolates of PVS to tubers would be limited to the early part of the season and become more limited with the onset of mature plant resistance, thus reducing reinfection in seed-lots in European production areas even when inoculum was present. By contrast, the lack of mature plant resistance to Minnesota isolates of PVS would allow spread from infected plants to tubers throughout the season, especially late in the growing season when contact between foliage and stems became more pronounced and the likelihood of mechanical transmission from plant to plant was increased. Weidemann (1986) also reported that not all tubers from an infected plant were infected after harvest and that the proportion of tubers infected was higher for plants infected early than those in which infections were detected later. Dedic (1978) noted that 12-14 days following artificial inoculations of the top leaves of potato with PVS, the percentage of tuber infection was 18.5-25% in cv. Jara and only 6.2% in cv. Krasava after 21 days. Tuber infection was not detected in cv. Krasava following inoculation of plants more than 6 weeks

old. It is not currently know whether strains of PVS in Tasmania are subject to mature plant resistance in local cultivars.

Primary infections of PVS were not detected by DAS-ELISA within the season during a trial conducted to assess transmission of PVS between potato plants (Chapter 5). Healthy potato plants were either caged or uncaged and with or without PVS infected potato plants. Where no infected source plants were placed either in caged on non-caged treatments PVS was not detected in potato plants. However, a high incidence of PVS was reported in plants exposed to infected source plants in both caged and non-caged treatments, indicating mechanical transmission and providing no evidence of aphid transmission. Interestingly PVS-source plants in this trial were not exposed to the extent of damage and wind-rub of field-grown plants.

This study has been able to demonstrate that limited increase in the incidence of PVS and PVX occurs within the season in seed potato fields in Tasmania, indicating that agronomic practices in Tasmania are effective in minimising transmission of PVS and PVX during the growing season. However, consideration of possible late season infection needs to be addressed due to an indication of an increase of PVS and PVX virus incidence in tubers collected from fields post harvest. Furthermore in the four fields studied, weed hosts appeared unimportant in the epidemiology of PVS and PVX, with no suggestion of aphid transmission of PVS.

8. General discussion and suggestions for further research

PVS was found to be widespread across growing regions of Tasmania, occurring in 66.7% of a range of generations of seed potato crops tested in 2002 season and 42% of G2 crops tested in 2003 season. PVS occurred at high incidence (>50%) in some crops and many potato cultivars, with 58.2% and 36.5% above the 1% tolerance of the National Standards for Certification of Seed Potato in 2002 and 2003 growing seasons respectively. For the 2002 season PVX was moderately prevalent in Tasmanian seed crops, occurred in 12.9% of crops tested, with 8.2% crops above the 1% tolerance of the National Standards. In G2 crops tested during the 2003 season PVX was less prevalent, occurring in 4.7% crops. The occurrence of these viruses above the 1% tolerance of the National Standards for Certification of Seed Potato results in prevention of certification and export of infected lines either interstate or internationally. Regional differences were observed with the mean incidence of PVS higher in the North East region than other parts of Tasmania. PVX was less prevalent and restricted to the regions of the North West coast, with most infected crops having low incidence. PVX was detected in a limited number of cultivars. In addition a study of PVS incidence in different generations tended to show a lower PVS incidence in earlier generation compared to later generations, suggesting virus spread may occur during subsequent growing seasons. The certification scheme currently requires only G0 crops to be routinely virus tested, while G1 – G5 crops are virus tested only when visual symptoms are observed in the field. Latent viruses, such as PVS, and occasionally PVX, show no or limited visual symptoms in the field, and therefore are not likely to be detected under certification standards based on visual

inspection. Relaxation in the past of standards required for seed potato in Tasmania may have contributed to the apparent increase in virus incidence in seed potato grown in Tasmania.

It is not readily apparent if PVS has been present in the seed industry for sometime or if PVS has been reintroduced recently and reached low to moderate incidence and prevalence. The introduction of the Tasmanian Certified Seed Potato scheme during the 1920s was primarily a management tool for controlling tuber borne pathogens, however, the scheme was also an effective way of reducing virus incidence. Briefly, the establishment of Tewkesbury Potato Station in 1933 enabled the production of mother seed, in isolation and at high altitude (>220 metres) to reduce the risk of virus infection from other potentially infected potato crops and avoid potential aphid vectors. In addition to ensure mother seed was virus free, heat therapy was introduced in the 1960s. With the establishment of a mini-tuber production facility at the Department of Primary Industries and Water in 1988, the industry experienced a relaxation of altitude requirements. This enabled early generation seed potato to be grown initially at 180 metres (previous requirement of 220 metres) and then below 180 m with ELISA testing for PLRV (Taylor 2003). Regardless of the changes to altitude restrictions PLRV has remained at low incidence in recent years, suggesting aphid activity may not be a major contributor to virus transmission in Tasmania. Although some strains of PVS are reportedly aphid transmissible, the low level of PLRV in the state would suggest aphids do not play a major role in virus transmission and other factors may explain the apparent increase in PVS incidence in recent years. However, caution is required as PLRV and PVS have

quite different modes of aphid transmission, i.e. persistent and non-persistent respectively, which may also explain differences in incidence. Similarly, PLRV produces obvious symptoms in potato, allowing infected plants to be selectively rogued out of seed crops, or highly infected crops to be removed from the seed scheme. This may have helped to maintain PLRV at low levels. By contrast PVS is latent in potato crops, and incidence can increase without being noticed in the absence of mandatory laboratory testing.

Twenty isolates of PVS were characterised as PVS^{O}-like based on local lesions on inoculated leaves of *C. quinoa*, serological detection in inoculated leaves only, and absence of symptoms or serological detection in non-inoculated leaves. A further three isolates were identified as PVS^{A}-like based on local lesions on inoculated leaves of *C. quinoa* and mild mottle and/or chlorotic spots on non-inoculated leaves, with virus detected by ELISA in inoculated and non-inoculated leaves. Fourteen PVS^{O}-like isolates produced no symptoms *C. quinoa*, and inoculated leaves only tested positive by ELISA. Nine isolates were PVS^{A}-like. Of these, four isolates produced no symptoms but tested positive by ELISA in inoculated and non-inoculated leaves and five isolates produced symptoms in inoculated leaves only but tested positive by ELISA in inoculated and non-inoculated leaves on *C. quinoa*. Subsequent analysis of 21 isolates by RT-PCR-RFLP, including isolates identified as PVS^{A} on *C. quinoa*, demonstrated RFLP patterns predicted for PVS^{O}. Furthermore, unlike characterised PVS^{A} strains, none of 23 Tasmanian PVS isolates were able to infect *L. esculentum*. While the RT-PCR-RFLP technique was unable to be verified due to the lack of a known PVS^{A} strain, results suggest that the

differentiation of PVS into only two strains may need revision and there is a need for a more in-depth study of the phylogeny and biological properties of PVS worldwide. Thirteen PVS isolates were successfully inoculated to *S. laciniatum*, but were symptomless. *Solanum laciniatum* has previously been reported as a host of PVA, PVX and PVY, but not PVS. *Solanum lacinatum* is sometimes observed growing as a weed in close proximity to fields in northern Tasmania. Although not common, its perennial nature may be a means by which PVS perennates.

Mechanised seed cutters were introduced into Tasmania in 1983 (B. Beattie, *personal communication*) followed soon after by commercial centralised seed cutters in the mid 1980s (B. Beattie, *personal communication*). This may have contributed to the apparent increase in PVS incidence in recent years. However, transmission studies demonstrated limited or no transmission of PVS on knives used to cut infected then healthy tubers. These findings are at odds with overseas studies that demonstrate seed cutting to be an efficient means of PVS transmission (Franc and Bantarri 1984). Results may suggest that strains of PVS within Tasmanian seed potato are not as readily transmissible by seed cutting as strains used in international studies and may require significant amounts of infected sap (e.g. on mechanical seed cutter) for PVS transmission to occur. If centralised seed cutters are important in facilitating virus transmission in Tasmania then other viruses such as PVX might also be expected to occur at higher incidence. However, unlike the widespread nature of PVS in seed crops, PVX was restricted to a limited number of crops and generally at low incidence. Symptom expression of PVX (although often mild) may be detected during visual inspection of the crops by seed certification officers or growers

and result in the rouging of infected plants possibly contributing to the low PVX incidence in crops in comparison to PVS. Alternatively PVX may be a more recent introduction into the seed scheme in Tasmania in comparison to PVS and have not had sufficient time to increase to the extent of PVS.

Trials to assess the effect of PVS on yield in cv. Russet Burbank grown in Tasmania suggested a 10% yield loss in plots with 100% PVS incidence compared to plots with no PVS. Although regression analysis led to low coefficient of determination, the negative linear relationship between PVS incidence and yield was highly significant. Regression analysis of plots demonstrated a difference between completely PVS infected plots and healthy plots of 5.6 t/ha, 6.3t/ha, and 10.1 t/ha, in 2002, 2003 and 2004 seasons, respectively. In Tasmania this equates to an economic penalty of between $1,500-3,000/ha for the grower. Findings from these yield studies in Tasmania are consistent with overseas studies where PVS has been shown to cause yield losses of up to 15% (Stevenson *et al.* 2001). In addition, results suggest a reduction in tubers size of those plots which were completely infected with PVS compared to healthy plots, which may contribute to the reduced yields. These results highlight concerns for the Tasmanian seed potato industry that PVS may result in potential yield loss and emphasize the importance of maintaining low PVS incidence in potato crops.

Spatial and temporal distribution of PVS and PVX in seed potato (cv. Russet Burbank) in Tasmanian in two trials within each of four commercial fields indicated limited spread was detected within crops during the season. An increase in PVS incidence during the

season of 5.2% and 25.5% was detected in one of four plot trials and one of four transect trials respectively, and 10.1% in incidence of PVX in one of two transect trials. Findings in this study are unlike those of many international studies (Cockerham 1958; Fletcher 1984; Fletcher *et al.* 1996; Franc and Banttari 2001; Kamenikova 1978) which have shown significant in-season spread. However, direct comparison of my study with international studies is difficult. A random distribution of infected seed pieces at planting was indicated by spatial analyses (SADIE) of PVS in plots. Although aggregation of PVS infected plants was detected by ORA in one field along transects suggesting some mechanical transmission of PVS had occurred between tubers during seed-cutting or during planting, no further spread was detected during the season. Random distributions were detected by SADIE and ORA at all times for PVX in plots and transect trials. Weed hosts and aphids appeared unimportant in the epidemiology of both viruses, and PVS respectively.

The prevalence and incidence of PVS and PVX in Tasmania seed potato identified by this project highlights the necessity for the Tasmanian seed potato industry to adopt control strategies to enable eradication of PVS and PVX from Tasmanian seed potato. As a result of this project laboratory testing is now currently conducted routinely on G2 seed crops in Tasmania for these viruses and provides added insurance to purchasers that seed potato sent from Tasmania for sale interstate meets the Certification criteria. In many other Australian states, laboratory testing is not practiced.

While monitoring can provide useful information on virus incidence, the spread of viruses throughout the State can only be controlled through the implementation of a management plan. This requires a series of measures to limit the spread and reinfection of certified seed stocks. The complexity and extent of any management program will depend on several factors including i) the economic penalty which could result from high levels of crop infection, ii) the virus tolerances imposed by the National Standards for Seed Certification and iii) the types of virus being detected and their mode of transmission.

The basis of the control of viruses is strict adherence to the National Standard for Certification of Seed Potato. This is based on a flush through, limited generation mode of seed potato production that has been successful in reducing virus problems in potato worldwide. Further steps to limit the spread of viruses, which could be implemented with industry agreement, include crop isolation, adoption of additional hygiene practices, cultural practices and quarantine issues and are discussed below.

The National Standard requires some isolation of G1 and G2 generation crops from later generation crops. However, isolation rules in the standard suggest only one blank row is required between generations, this would allow contact between plants of different generations after row closure. Greenhouse studies in this project demonstrated transmission of PVS to healthy potato plants in close contact with PVS infected plants. This suggests wind-rub damage would allow PVS transmission in field plants and suggests a greater number of blank rows (3-4 rows) are required to minimise potential virus transmission between infected and non-infected generations. In Western Australia

and Victoria three and two blank rows are recommended, respectively. Complete isolation of G1 crops from other seed crops would be preferable to reduce the risk of not only mechanical transmission between infected and non-infected plants but also reduce the potential risk of aphid transmission of PVS. However, it is not known if strains of PVS in Tasmania are aphid transmissible. Similarly a greater number of blank rows (eg. 3-4 blank rows) between different generations of seed crops is required to minimise virus transmission as the National Standard currently requires only 1 blank row. The growing of seed crops in close proximity to ware crops is likely to have increased the potential for mechanical transmission into early generation seed. In addition a greater distance between seed and ware crops would be preferable as currently the National Standard requires only 20 metres, which would not provide an effective barrier for potential aphid flights. Given the high incidence of PVS detected recently in Tasmanian seed crops it would be preferable to increase this gap to 50-100 metres or more. In addition planting a barrier crop around the perimeter of the seed potato crop would provide protection from non-persistent aphid transmission. However, the National Standard currently promotes bare soil around the perimeter of generations of seed crops.

Strict hygiene requirements are necessary to ensure that low generation seed is not graded or cut at the same facilities as seed of high generation or ware crops as this can facilitate virus transmission. In practice this is difficult for sectors of the industry to avoid, in particular where centralised cool stores and seed cutting operations are used. Although the National Standard recommends disinfestation of equipment during seed cutting operations, especially between lines, it is often impractical for commercial seed cutting operations due to the cost of shut down and downtime. Alternatively if seed pieces were

to be disinfested with an antiviral agent (eg Virkon® S or Viraclean) after the cutting process to eliminate virus transmission after cutting this may make cutting operations more cost effective with less scheduled shut downs. Our study was unable to fully assess the ability of antiviral chemicals to disinfest tubers after seed cutting. Further investigation is required to assess this as a potential virus control strategy. The National Standard recommends regular disinfestation of cutting equipment between and also within lines when cutting seed by hand. The National Standard promotes bin hygiene and introduction of a bin cleanliness certificate and the use of plastic bins, which are easier to clean. This would help to reduce the transmission of PVS and PVX which can be retained on such surfaces for some time. Although others (eg. Banttari *et al.* 1978; Banttari *et al.* 1993) have conducted studies on the retention of infectivity by PVS and PVX on various materials, recent studies are lacking. This is particularly the case for Tasmanian strains of PVS in order to determine alternative sources of inoculum that may be contributing to the high PVS incidence in Tasmania.

The National Standard also suggests staff of companies and seed certification staff and to enforce a strict hygiene plan between each crop visited. Tasmanian seed certification staff have adopted a hygiene plan that includes wearing disposable boot covers in each seed potato field and disinfecting hands and spraying clothing with disinfectant between seed potato fields. In addition, most field inspections are conducted before full row closure to reduce crop damage and potential for sap transmission between plants. Another important aspect of farm hygiene outlined by the National Standard is the development of in-field wash down procedures and disinfesting machinery between generations or

different crops. Cultural aspects include conducting machinery operations in order of generation, i.e. from G1 to G5, as later generations would have had a longer exposure period to potential virus infection, and thus pose a potential source of inoculum for virus transmission to early generation crops, facilitated by mechanical transmission. Thus restricting the movement of machinery through the crop would help to avoid mechanical transmission of viruses. In Tasmania aerial spraying is a routinely employed cultural practice adopted by Tasmanian potato seed growers to minimise traffic in the crop. Irrigation practices in Tasmania typically include the use of centre pivot irrigator introduced in the early 1990s, gun irrigators and solid set irrigation. Removal of plants from wheel tracks of centre pivot irrigator and gun irrigators is necessary to avoid mechanical transmission of virus in the crop. However, many centre pivot wheel tracks are not necessarily cleared of plant material and so there is potential for virus spread as they move through the crop. Other strategies to avoid virus transmission include cutting and planting seed before sprout formation which is associated with increased virus concentrations in the tuber and potential for damage to sprouts, both of which increase potential for transmission of mechanically transmitted viruses to seed tubers during cutting and planting operations.

The National Standards recommends strict control of volunteer potatoes and weeds (especially nightshade and fathen) that may be reservoirs of vectors and/or viruses. Control of volunteer potato is a major issue for the Tasmanian industry and there is potential for virus to survive between periods of rotation in this manner. Although a minimum five-year rotation of seed potato is required in Tasmania, unfortunately this is

insufficient under Tasmanian conditions to completely eradicate volunteer potato from the previous crop, potentially allowing PVS to enter the succeeding crop. Other seed schemes such as the Seed Potato Classification Scheme in Scotland require pre-basic crops (equivalent to G0) to be grown in land on which potatoes have not been grown for the previous seven years (Anon. 2007). A longer rotation period to reduce the risk of volunteer potato providing potential virus reservoirs is not commercial viable in Tasmania. Although annual weeds did not appear to be important in the spread of PVS and PVX in trials conducted in Tasmania, PVS was detected by DAS-ELISA in a number of weed hosts including *Malva syvestris* and *Rumex obtusifolius* (Chapter 7). In addition, perennial weeds hosts may provide an overwintering host for aphids and a potential virus host for PVS. As mentioned previously Tasmanian PVS isolated were able to infect *S. laciniatum*. Further work is necessary to determine if *S. laciniatum* is naturally infected by PVS and PVX in the field and whether it is a host for over wintering aphid vectors of PVS. In addition further research is required to determine if Tasmanian strains of PVS are aphid transmissible to *S. laciniatum*. Further work to investigate other potential perennial weed hosts of PVS in Tasmania is required. In addition studies are required to assess the potential role of aphid transmission of PVS in Tasmanian seed potato, to determine if Tasmanian PVS isolates are aphid transmissible. The National Standards suggest control of vectors of vector transmitted viruses through e.g. isolation of crops, chemical control, removal of alternative hosts and adjustment of planting date to avoid vector flights will aid in low virus incidence.

Quarantine issues include removal of seed crops with a greater than 1% level of virus from the seed scheme, e.g. by processing tubers. For PVS, this would require routine laboratory testing to identify infected crops. There is also a need to restrict the movement of seed that has not been virus tested between regions given the high incidence of PVS and PVX in some regions of Tasmania.

Additional protocols that may be adopted by the Tasmanian seed potato industry include tuber testing as is carried out in USA after harvest. In USA this involves a grow out test over winter followed by laboratory testing for virus in sprout or leaf material. Samples comprising 300-800 small seed tubers (30-60 g each) are collected for each seed lot, dormancy broken and then planted in warm climate regions (eg. California and Florida) to promote expression of virus symptoms (Slack and Singh 1998). Under some USA seed potato schemes, diseased tubers are determined by expression of symptoms, however, this is not effective for determining infection of latent viruses such as PVS and PVX. In other schemes in the USA, mandatory laboratory testing of early generations and voluntary testing of later generations is utilised. Due to potential issues of detecting virus at low titre by ELISA in tubers a more reliable and sensitive molecular test such as RT-PCR may be required.

In conclusion it seems from the prevalent nature and reported high incidence of PVS in Tasmanian seed potato that this virus may not be readily eliminated from the seed certification scheme without a much more stringent management strategy than has been currently adopted. Conversely the lower prevalence and incidence of PVX suggests that

this virus will be more easily eradicated from the seed scheme. Results from this project indicate that although numerous overseas studies have been conducted on many aspects of PVS including modes of transmission, effect on yield and spread of PVS in the field, there is much still to learn about the epidemiology of this virus in Tasmania.

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Appendix I: Previous publications from this project

Symposium paper:

Lambert, S.J., Hay, F.S., Pethybridge, S.J., and Wilson, C.R. Spatiotemporal spread of *Potato virus S* and *Potato virus X* in seed potato in Tasmania, Australia. *Plant Health Progress* (in press).

Conference posters:

Lambert, S., Hay, F., Pethybridge, S., and Wilson, C. (2005). Spread of *Potato carlavirus* S (PVS) and *Potato potexvirus* X (PVX) in seed potato in Tasmanian, Australia. *IX International Plant Virus Epidemiology Symposium*. International Potato Center. Lima, Peru. 4-7 April 2005.

Lambert, S., Hay, F., Kirkwood, I., Pethybridge, S., Cross, P., and Wilson, C. (2005). Prevalence and incidence of *Potato carlavirus* S (PVS) and *Potato potexvirus* X (PVX) in Tasmanian seed potato. *2nd Joint Conference of the International Working Groups on Legume (IWGLV) and Vegetable Viruses (IWGVV)*. Hosted by the University of Florida. Fort Lauderdale, Florida, U.S.A. 10-14 April 2005.

Reports:

Lambert, S. (2005). Assessment of the rate and means of spread of *Potato carlavirus* S (PVS) and *Potato potexvirus* X (PVX) in seed potato in Tasmanian, Australia. *2004 AFFA Science and Innovation Awards for Young People.* Department of Agriculture, Fisheries and Forestry, Bureau of Rural Sciences, Canberra, Australia.

Hay, F., Kirkwood, I., Lambert, S., Cross, P. Wilson, C., and Pethybridge, S. (2004) A survey to determine the prevalence and incidence of common viruses in potato seed stocks in Tasmania. Final Report for Project PT02037 (March 2004). Horticulture Australia Ltd.

Hay, F., Lambert, S., Kirkwood, I., Cross, P. Wilson, C., and Pethybridge, S. (2005) Management strategy for elimination of virus from certified seed potato stocks in Tasmania. Final Report for Project PT03069 (April 2005). Horticulture Australia Ltd.

Hay, F., Lambert, S., Kirkwood, I., Cross, P. Wilson, C., and Pethybridge, S. (2006) Managing viruses in Tasmanian seed potato stocks. Final Report for Project PT05011 (December 2006). Horticulture Australia Ltd.

Appendix II: Serological testing, double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

Virus testing was conducted at Tasmanian Institute of Agricultural Research, University of Tasmania, Cradle Coast Campus, Burnie, Tasmania unless otherwise stated. Antisera for PVS and PVX was obtained from Agdia Inc. Elkart, IN, USA. A double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (Clark and Adams 1977) was used to serologically test samples for PVS and PVX infection. Antiserum was pipetted into 96 well microtitre plates (Nunc) at 1/300 dilution (PVS) and 1/500 dilution (PVX) in carbonate coating buffer (1.59 g Na_2CO_3 and 2.93 g $NaHCO_3$, made up to 1 L with distilled water and adjusted to pH 9.6). A total of 100 µl was added to each well. Microtitre plates were incubated at 37°C for 4 hours or at 4°C overnight and washed (below).

Preparation of leaf samples included the top half of each leaf sample being cut off at right angles to the veins and discarded. A further two cuts were made on each side of the vein at 45° angle to form a blunt arrowhead shape. A strip (2-3 mm wide and 1-3 cm long) was cut from the cut surface running at right angles to the veins. Scissors were sterilised in 10% household bleach solution and wiped clean between each individual leaf. Sap was expressed from leaflets using a motorised roller press. A stock solution (10x) of Phosphate Buffered Saline (PBS), 10X, contained 80 g NaCl, 2.0 g KH_2PO_4, 14.413 g $Na_2HPO_4.2H_2O$ and 2.0g KCl, made up to 1 L with distilled water and adjusted to pH 7.4. Extraction buffer (100 ml PBS 10X, 1.3 g anhydrous sodium sulfite, 20.0g

Polyvinylpyrrolidine (MW 24-40,000), 2.0 g Bovine Serum Albumin, 20 ml Tween 20, made up to 1 L and adjusted to pH 7.4) was run down the rollers and sap and extraction buffer collected in a 1 ml sample tube. Rollers were washed with tap water between samples to prevent cross-contamination of samples.

Sap samples prepared as above were pipetted into wells (100 µl/well) and incubated overnight at 4°C. Samples were added to plates, with wells containing 4 known negative samples, buffer only and a positive sample. Antiserum conjugated with alkaline phosphatase was prepared for each virus at the same dilution used to coat the plate. Conjugated antiserum was diluted in conjugation buffer (100 ml PBS 10x, 2.0 g Bovine serum albumin, 20.0 g Polyvinylpyrrolidine (MW 24-40,000), made up to 1 L with distilled water and adjusted to pH 7.4). To each well, 100 µl dilute conjugated antiserum was added and microtitre plates incubated at 37°C for four hours. Plates were washed (below). Substrate tablets, each containing 5 mg p-nitrophenyl phosphate were added at a rate of 1 tablet per 10 ml of substrate buffer, (48.5 ml Diethanolamine and 400 ml of distilled water adjusted to pH 9.8). Substrate (100 µl) was added to each well. Plates were incubated for 30-60 minutes at room temperature to allow colour development and absorbance of each well was read at 405 nm using a Titertek photometer (Flow Laboratories, Helsinki, Finland). Samples were considered positive if they were greater than the mean absorbance of the negative controls plus three times the standard deviation of the negative controls (Sutula *et al.* 1986).

Microtitre plates were washed between each step with three changes of wash buffer (100 ml PBS 10x stock, 900 ml distilled water, 0.5 ml Tween 20 and 1.0g milk powder, made up to 1 L and adjusted to pH 7.4). Plates were immersed in wash buffer to fill wells and soaked for at least three minutes between each change. After the final soak, plates were emptied of wash buffer, and allowed to drain upside down over paper towels for approximately five minutes before the next step.

Appendix III: Weather data collected during the monitoring of spatial patterns of *Potato virus S* (PVS) and *Potato virus X* (PVX)

Appendix IIIa Weather collected during the monitoring of spatial patterns of *Potato virus S* (PVS) and *Potato virus X* (PVX) during a field trial in 2004 (Field 1, Riana, Tasmania).

Date	Air temperature within canopy (*C)			Relative humidity (%)			Rainfall
(d/mm/yy)	min.	mean	max.	min.	mean	max	(mm) [1]
06/01/04	11.7	16.4	21.7	20.7	37.5	85.7	9.40
07/01/04	5.3	12.0	18.3	29.2	69.1	95.4	4.31
08/01/04	3.6	9.3	16	29.2	62.9	97.1	2.52
09/01/04	8.2	12.8	18.3	32.0	50.0	87.3	11.18
10/01/04	10.9	13.8	18.3	37.9	70.5	94.7	3.54
11/01/04	6.5	11.8	19	23.5	54.1	87.8	0.76
12/01/04	7.3	14.3	23.7	22.2	37.5	50.4	11.68
13/01/04	8.6	12.9	17.9	35.6	58.5	87.8	1.52
14/01/04	5.7	10.9	17.5	26.4	39.6	54.3	0.00
15/01/04	6.1	13.6	21	23.5	37.8	49.1	0.00
16/01/04	7.3	14.3	21.7	23.5	37.4	47.7	0.76
17/01/04	6.9	11.2	16.4	26.4	47.7	83.5	0.00
18/01/04	4.5	10.0	16.4	26.4	37.8	51.9	0.00
19/01/04	5.7	11.9	18.7	25.0	38.7	48.4	3.30
20/01/04	9.8	15.3	21.3	26.4	52.9	83.5	0.25
21/01/04	4.5	11.2	17.1	28.3	41.0	50.1	0.00
22/01/04	7.7	11.3	15.2	26.4	38.8	49.3	1.01
23/01/04	4.0	11.3	17.5	30.1	42.9	55.9	0.25
24/01/04	7.7	13.5	19	25.0	36.4	46.9	0.00
25/01/04	11.7	14.4	19	25.0	36.1	43.7	0.76
26/01/04	11.3	14.2	18.3	31.1	50.9	75.4	0.25
27/01/04	8.6	14.5	19.4	26.4	43.2	62.8	1.76
28/01/04	11.3	13.1	14.8	34.6	55.8	85.2	1.77
29/01/04	12.1	12.8	14.1	43.7	59.6	79.6	0.50
30/01/04	11.3	15.2	19.8	31.1	55.7	81.2	0.00
31/01/04	10.1	15.2	20.6	25.0	38.3	54.0	0.00
01/02/04	11.3	15.5	19.8	25.0	38.0	60.5	0.00
02/02/04	12.1	15.8	21	20.7	30.9	38.8	0.00
03/02/04	9.4	14.5	20.6	22.2	29.2	37.0	0.00

Appendix IIIa cont.

Date	Air temperature within canopy (*C)			Relative humidity (%)			Rainfall
(d/mm/yy)	min.	mean	max.	min.	mean	max	(mm) [1]
04/02/04	9.4	14.3	20.2	23.5	33.1	41.8	0.25
05/02/04	7.7	14.5	21.0	33.0	42.8	62.8	0.00
06/02/04	8.6	14.3	20.2	20.7	34.2	44.1	3.56
07/02/04	7.7	13.2	19.0	25.0	36.5	45.6	5.78
08/02/04	12.1	15.7	21.0	30.1	37.4	43.2	0.50
09/02/04	7.7	13.6	18.7	22.2	30.5	42.0	2.29
10/02/04	7.3	12.5	17.9	35.0	42.6	64.7	8.80
11/02/04	9.0	14.3	21.0	26.4	33.6	40.3	4.50
12/02/04	12.9	15.2	18.3	36.0	39.5	43.9	6.00
13/02/04	11.3	15.9	22.1	20.7	35.5	43.5	3.50
14/02/04	13.3	16.4	20.6	27.3	36.1	43.5	2.25
15/02/04	9.4	17.6	23.3	22.2	55.9	79.2	2.75
16/02/04	8.2	14.1	22.1	22.2	30.7	38.8	2.00
17/02/04	10.1	15.1	22.1	20.7	32.5	44.1	1.75
18/02/04	7.3	14.1	23.3	20.7	28.4	39.6	1.50
19/02/04	8.6	12.7	19.8	20.7	30.6	43.5	2.00
20/02/04	11.3	13.6	18.3	25.0	31.9	38.5	1.25
21/02/04	8.6	12.5	18.7	22.2	32.0	40.9	1.25
22/02/04	6.9	11.5	17.9	22.2	33.7	43.7	0.75
23/02/04	4.9	10.4	16.8	23.5	35.8	48.4	0.50
24/02/04	6.5	12.7	19.4	22.2	31.0	39.1	0.00
25/02/04	10.9	15.1	21.0	20.7	33.0	41.4	0.00
26/02/04	12.1	14.8	18.3	28.3	36.0	40.9	0.00
27/02/04	12.5	15.8	21.7	25.0	36.9	43.5	0.00
28/02/04	6.5	12.4	21.0	32.5	45.8	71.4	0.00
29/02/04	3.2	8.5	13.7	36.0	46.3	75.1	0.00
01/03/04	3.2	11.6	19.4	22.2	36.8	49.9	0.00
02/03/04	10.1	15.1	20.6	23.5	35.0	43.2	0.00
03/03/04	12.1	16.9	22.9	20.7	34.0	42.3	0.00
04/03/04	8.6	16.3	23.7	22.2	35.2	44.9	13.73
05/03/04	8.2	13.8	20.2	23.5	37.7	46.5	0.00
06/03/04	7.7	11.8	15.6	46.3	57.0	80.0	0.00
07/03/04	7.7	14.9	19.0	37.0	51.9	85.7	0.00
08/03/04	11.3	14.2	17.9	43.7	60.1	80.7	0.00
09/03/04	4.5	10.5	16.8	35.0	50.4	81.5	0.00
10/03/04	3.6	10.2	19.8	26.4	43.1	54.5	0.00
11/03/04	4.5	10.0	14.1	40.9	52.5	83.1	0.00
12/03/04	6.5	12.7	20.6	28.3	42.7	63.9	0.00
13/03/04	4.9	12.0	19.8	29.2	41.1	53.7	0.00

[1]total rainfall for the 24 hour period

Appendix IIIb Weather collected during the monitoring of spatial patterns of *Potato virus S* (PVS) and *Potato virus X* (PVX) during a field trial in 2004 (Field 2, Riana, Tasmania).

Date	Air temperature within canopy (*C)			Relative humidity (%)			Rainfall
(d/mm/yy)	min	mean	max	min	mean	max	(mm)[1,2]
09/01/04	15.6	21.8	26.8	31.1	47.0	53.2	0.00
10/01/04	19.4	20.3	21.3	51.7	53.1	54.0	0.00
11/01/04	18.3	19.5	21.0	45.8	50.6	53.2	0.00
12/01/04	18.3	20.2	22.9	43.2	46.2	48.0	0.00
13/01/04	13.7	19.8	25.2	34.0	46.7	54.5	0.00
14/01/04	6.1	14.0	23.7	29.2	47.2	77.3	0.00
15/01/04	5.7	15.3	26.8	36.6	73.5	99.5	0.00
16/01/04	4.0	16.1	29.1	35.6	72.0	100.0	0.00
17/01/04	4.9	13.1	23.3	29.2	66.7	94.7	0.00
18/01/04	2.7	11.6	23.3	22.2	62.2	96.2	0.00
19/01/04	0.1	13.1	26.8	31.1	68.8	100.0	0.00
20/01/04	10.1	18.5	31.6	22.2	67.1	98.7	0.00
21/01/04	1.0	12.5	24.8	36.0	74.3	99.5	0.00
22/01/04	4.5	13.4	23.7	25.0	66.2	100.0	0.00
23/01/04	1.9	15.4	32.0	20.7	65.2	99.5	0.00
24/01/04	3.2	14.0	25.6	44.9	77.7	100.0	0.54
25/01/04	9.8	15.2	23.7	53.5	83.2	97.1	0.02
26/01/04	10.9	16.7	28.0	42.3	80.9	100.0	0.01
27/01/04	7.7	17.4	29.1	38.8	80.0	98.7	0.20
28/01/04	11.3	14.3	19.4	80.7	95.8	100.0	0.41
29/01/04	12.1	13.6	16.4	98.7	99.7	100.0	0.31
30/01/04	10.5	16.5	28.0	60.7	92.6	100.0	0.04
31/01/04	7.7	15.3	24.8	64.7	91.1	100.0	0.01
01/02/04	7.7	15.5	26.0	61.7	90.9	100.0	0.34
02/02/04	10.5	16.2	28.0	50.9	87.8	100.0	0.25
03/02/04	6.9	15.3	26.8	48.2	79.0	99.5	0.01
04/02/04	6.1	14.3	22.9	62.6	83.5	98.7	0.00
05/02/04	6.5	15.0	24.8	65.4	87.1	98.7	0.64
06/02/04	9.8	16.0	25.6	48.2	76.5	97.1	0.00
07/02/04	6.5	13.9	22.5	61.5	86.0	97.9	0.00
08/02/04	12.1	16.4	23.3	83.5	94.3	99.5	0.01
09/02/04	7.3	13.3	19.4	61.2	82.3	95.4	0.00
10/02/04	4.5	11.6	19.4	71.6	89.4	99.5	0.00
11/02/04	7.7	14.5	22.5	68.8	86.1	98.7	0.00
12/02/04	12.1	15.3	19.4	92.3	97.1	99.5	0.00
13/02/04	7.3	15.7	24.8	50.9	82.9	100.0	0.00

Appendix IIIb cont.

Date	Air temperature within canopy (*C)			Relative humidity (%)			Rainfall
(d/mm/yy)	min	mean	max	min	mean	max	(mm)[1,2]
14/02/04	13.3	16.7	21.3	81.5	93.6	98.7	0.00
15/02/04	11.3	18.4	24.0	69.5	91.6	100.0	0.00
16/02/04	8.2	14.3	22.5	71.1	86.8	98.7	0.00
17/02/04	7.7	14.5	22.9	56.3	84.6	99.5	0.00
18/02/04	6.1	14.0	22.5	53.7	78.8	98.7	0.00
19/02/04	9.8	13.5	19.8	56.6	75.9	89.0	0.00
20/02/04	10.9	13.3	18.7	79.2	90.2	97.1	0.00
21/02/04	9.0	13.0	19.4	65.5	84.0	93.9	0.00
22/02/04	5.7	11.4	17.5	64.6	88.3	99.5	0.00
23/02/04	4.9	10.4	16.8	81.8	94.3	100.0	0.00
24/02/04	2.3	10.5	20.2	73.3	90.4	100.0	0.00
25/02/04	9.0	14.1	21.3	70.7	90.7	99.5	0.00
26/02/04	12.1	14.8	19.4	85.7	93.4	98.7	0.00
27/02/04	11.3	15.5	21.7	77.8	93.3	98.7	0.00
28/02/04	6.1	12.9	22.5	70.2	91.7	99.5	0.00
29/02/04	6.9	9.8	14.8	82.3	95.8	100.0	0.00
01/03/04	2.3	9.9	19.4	70.9	89.9	100.0	0.00
02/03/04	4.5	12.7	20.2	76.2	91.8	99.5	0.00
03/03/04	10.5	16.2	23.7	78.9	92.1	97.9	0.00
04/03/04	9.4	16.0	22.9	69.7	90.1	97.9	0.00
05/03/04	8.6	13.0	19.8	83.5	90.6	97.1	0.00
06/03/04	9.4	12.6	14.8	97.1	99.0	100.0	0.00
07/03/04	4.5	11.3	17.5	99.5	99.7	100.0	0.00
08/03/04	11.7	14.7	18.3	99.5	99.6	100.0	0.00
09/03/04	5.7	11.3	16.8	89.8	97.6	100.0	0.00
10/03/04	2.7	10.0	19.4	71.1	89.2	100.0	0.00
11/03/04	1.0	7.6	14.8	90.6	95.4	99.5	0.00
12/03/04	2.3	11.4	21.0	66.6	89.0	99.5	0.00
13/03/04	4.0	12.0	20.2	72.5	88.4	98.7	0.00
14/03/04	4.9	12.0	21.7	65.0	87.0	98.7	0.00
15/03/04	0.6	8.9	17.5	85.2	92.8	98.7	0.00
16/03/04	1.4	10.1	21.3	56.8	83.9	98.7	0.00
17/03/04	0.6	8.1	15.6	69.2	89.6	97.9	0.00
18/03/04	2.7	10.3	15.6	82.3	93.2	98.7	0.00
19/03/04	6.5	12.9	20.2	73.5	89.5	97.9	0.00
20/03/04	5.3	11.2	18.3	56.3	81.1	97.9	0.00
21/03/04	2.7	10.4	21.0	54.7	78.1	95.4	0.00
22/03/04	0.6	10.8	22.5	59.9	83.4	97.1	0.00
23/03/04	4.0	11.4	19.8	56.2	80.5	95.4	0.00
24/03/04	4.9	12.0	22.9	65.4	89.1	97.9	0.00
25/03/04	6.5	12.6	21.7	56.3	86.4	99.5	0.00

Appendix IIIb cont.

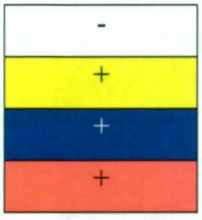
Date	Air temperature within canopy (*C)			Relative humidity (%)			Rainfall
(d/mm/yy)	min	mean	max	min	mean	max	(mm)[1,2]
26/03/04	5.3	11.2	19.8	64.7	86.4	97.9	0.00
27/03/04	1.4	10.6	23.3	52.9	80.9	98.7	0.00
28/03/04	9.4	11.9	17.5	80.0	92.3	95.4	0.00

[1]total rainfall for the 24 hour period
[2]datalogger was buried in foliage, rainfall may not be an accurate indicator

Appendix IV Distribution of PVS and PVX infected plants in transect trials

Appendix IV-1. (a,b), IV-2 (a,b), IV-3 (a,b) and IV-4 (a,b,c). A visual representation of *Potato virus S* (PVS) and *Potato virus X* (PVX) incidence in plot trials in seed potato (cv. Russet Burnbank) in Tasmania at field 1 (58 and 129 days after planting, DAP), field 2 (58 and 129 DAP), field 3 (31 and 107 DAP) and field 4 (30, 54 and 105 DAP) are shown in Figure IV(1-4), respectively during 2003 and 2004 growing seasons.

Key to Figures IV-1. (a,b), IV-2 (a,b), IV-3 (a,b) and IV-4 (a,b,c)

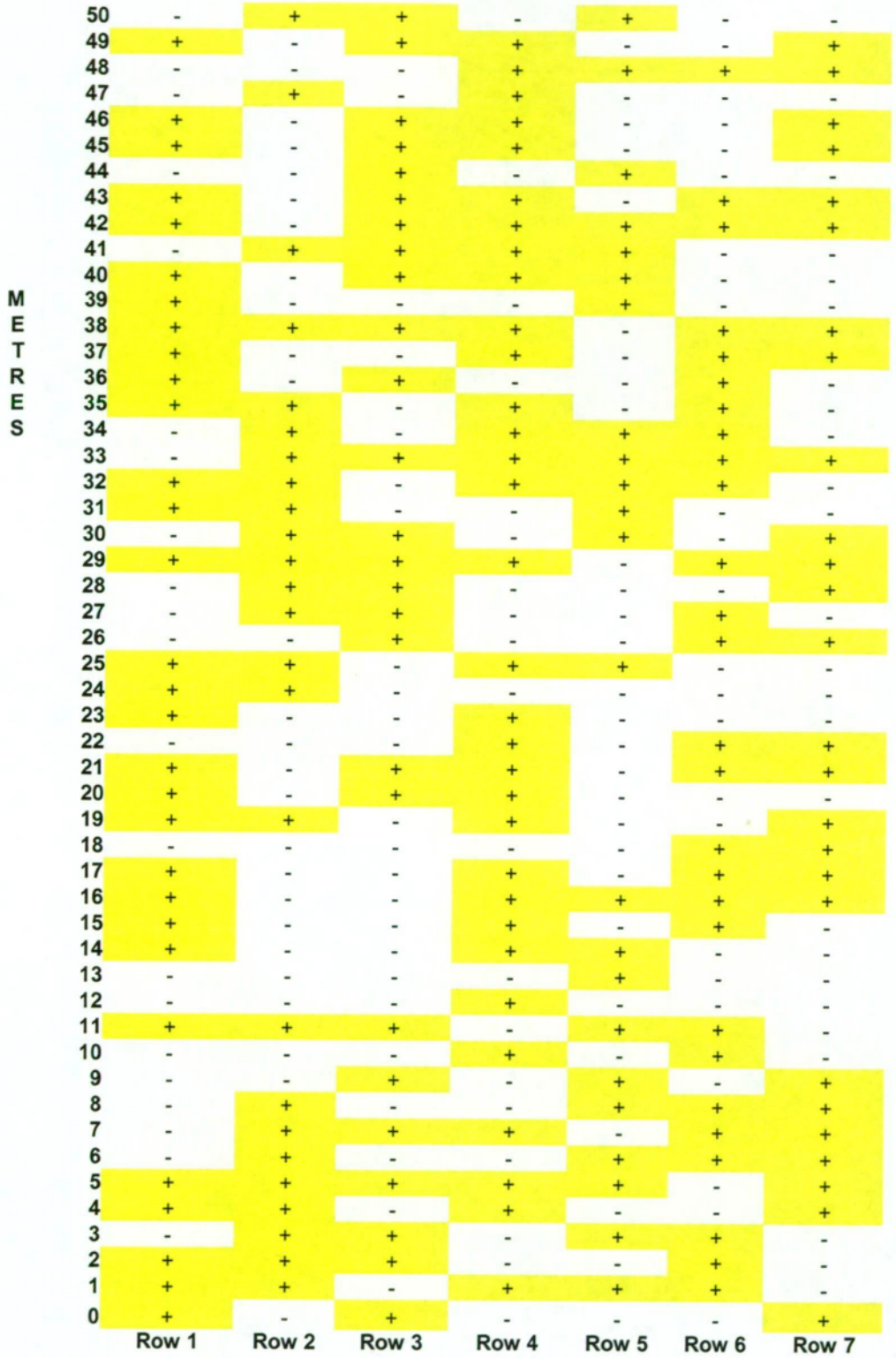


Healthy leaf

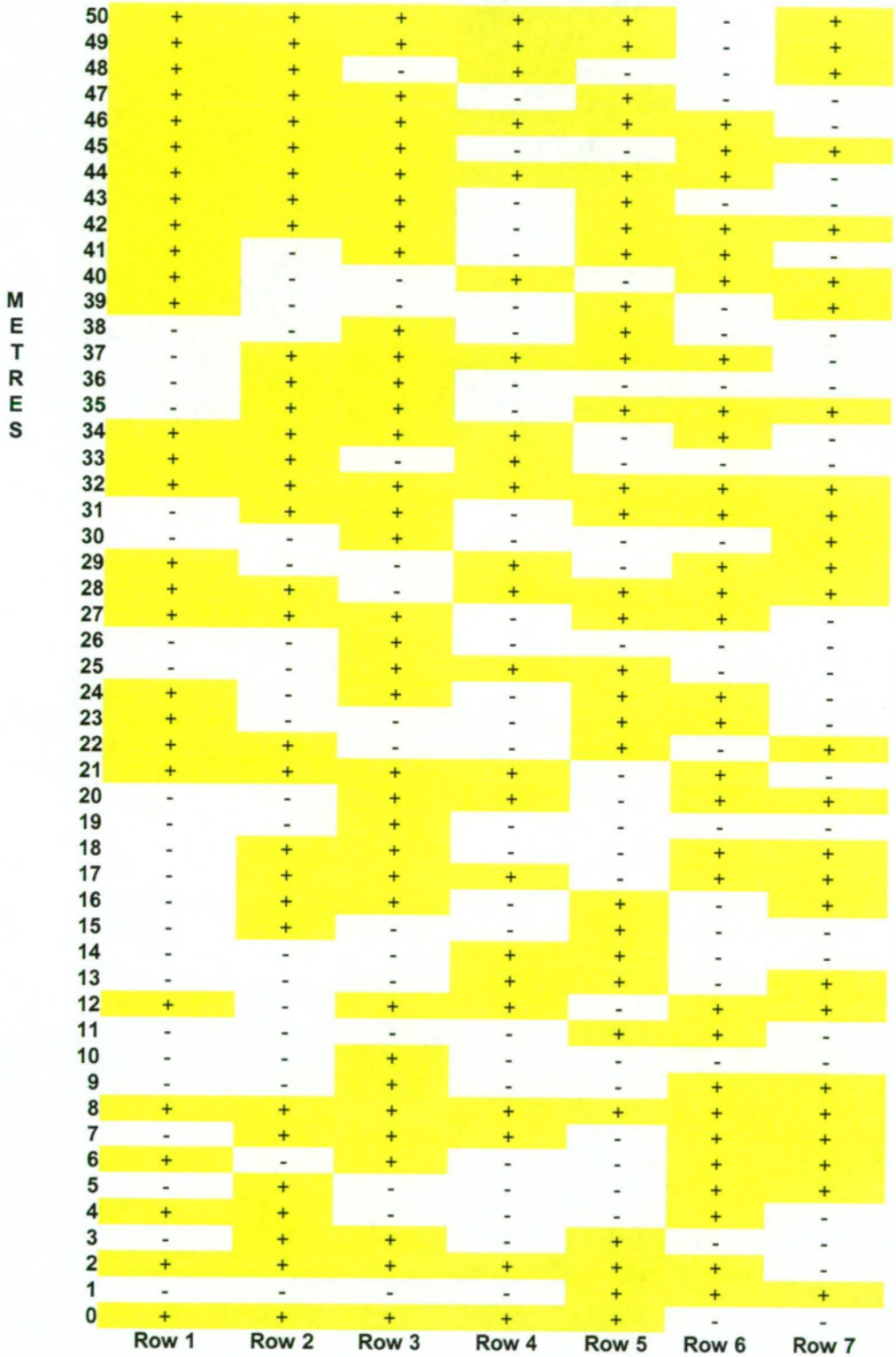
PVS infected leaf

PVX and PVS infected leaf

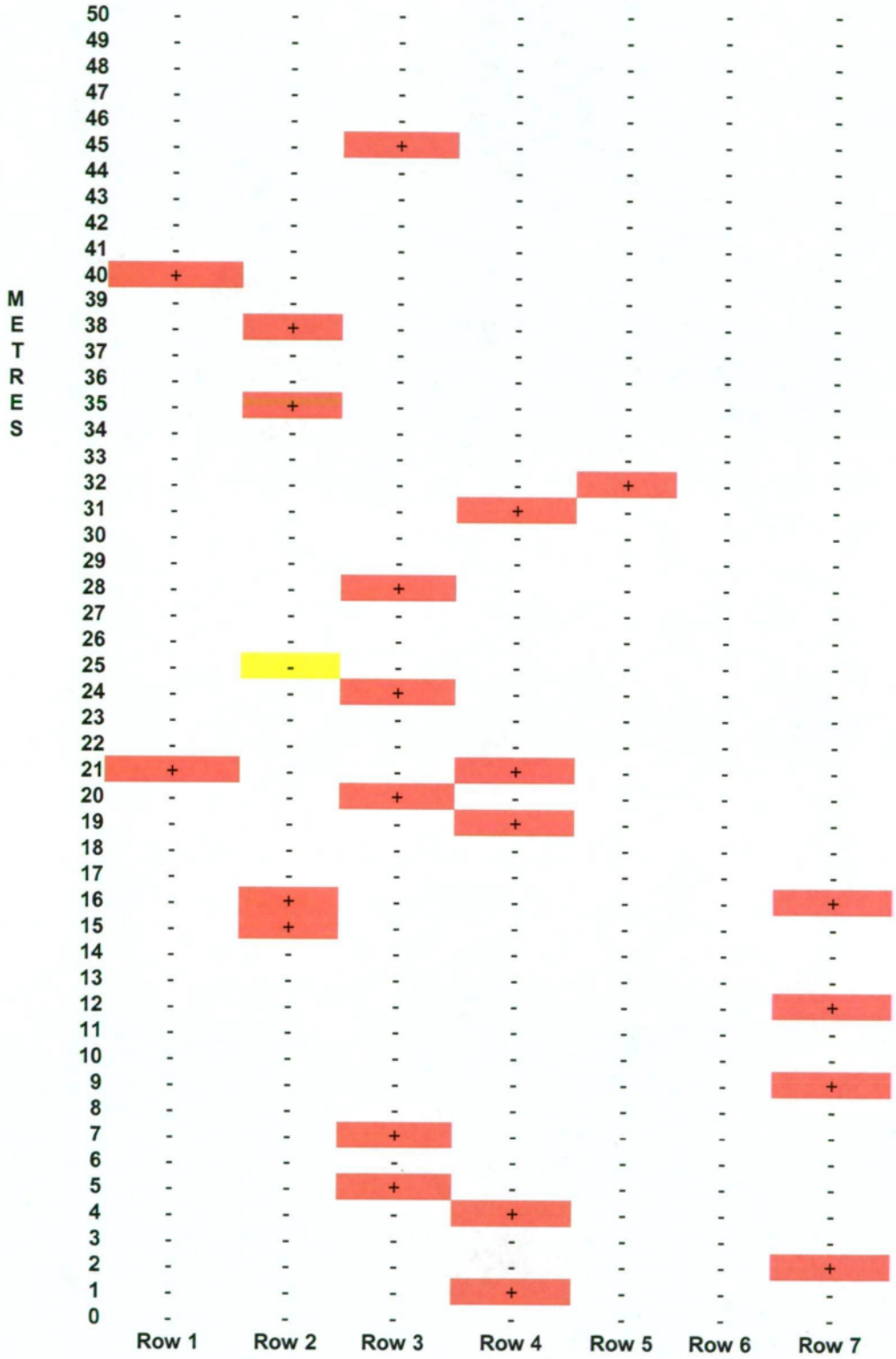
PVX infected leaf



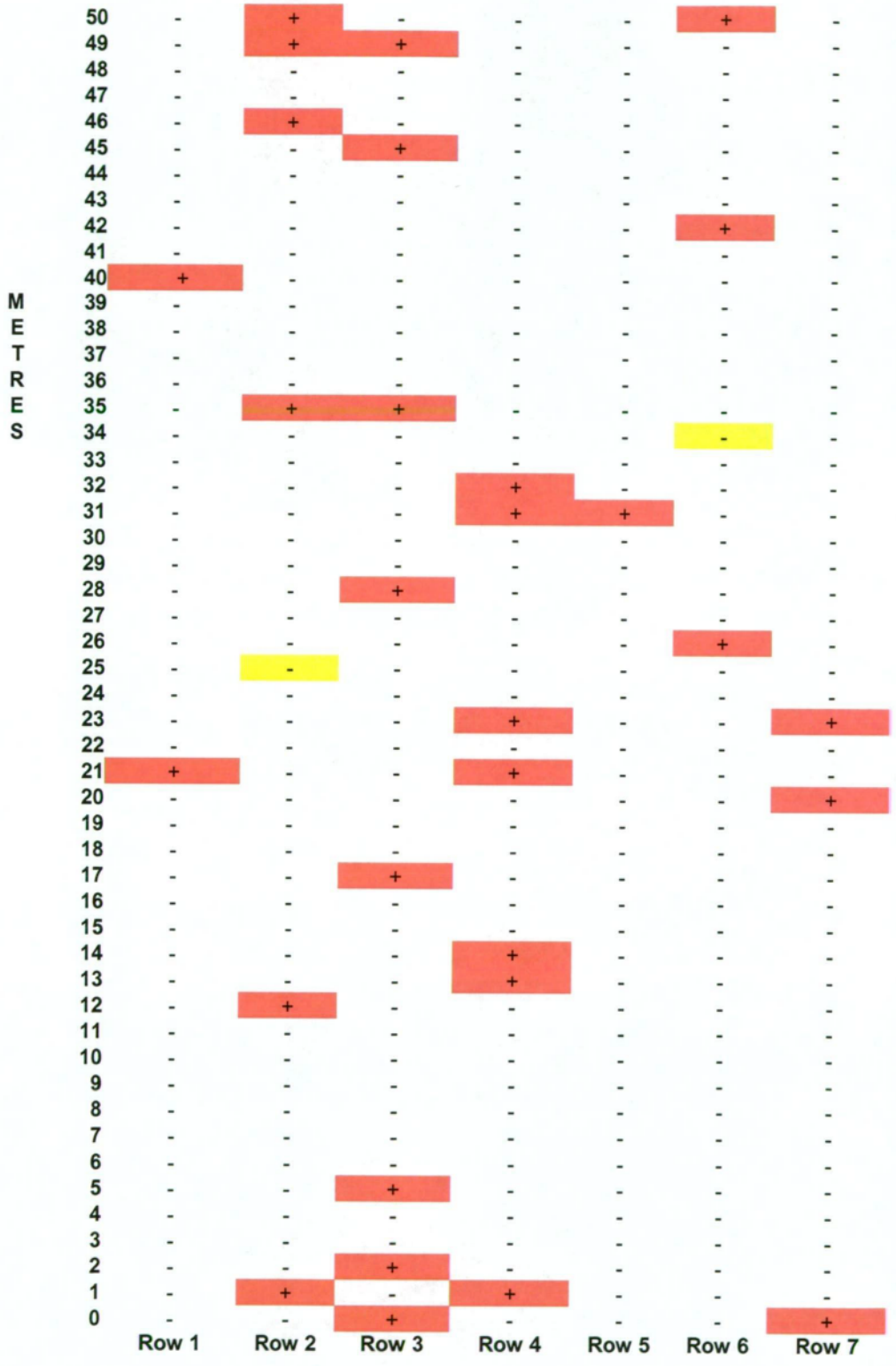
Appendix IV-1a. Map of PVS infection in transect trial at field 1 at 58 DAP.



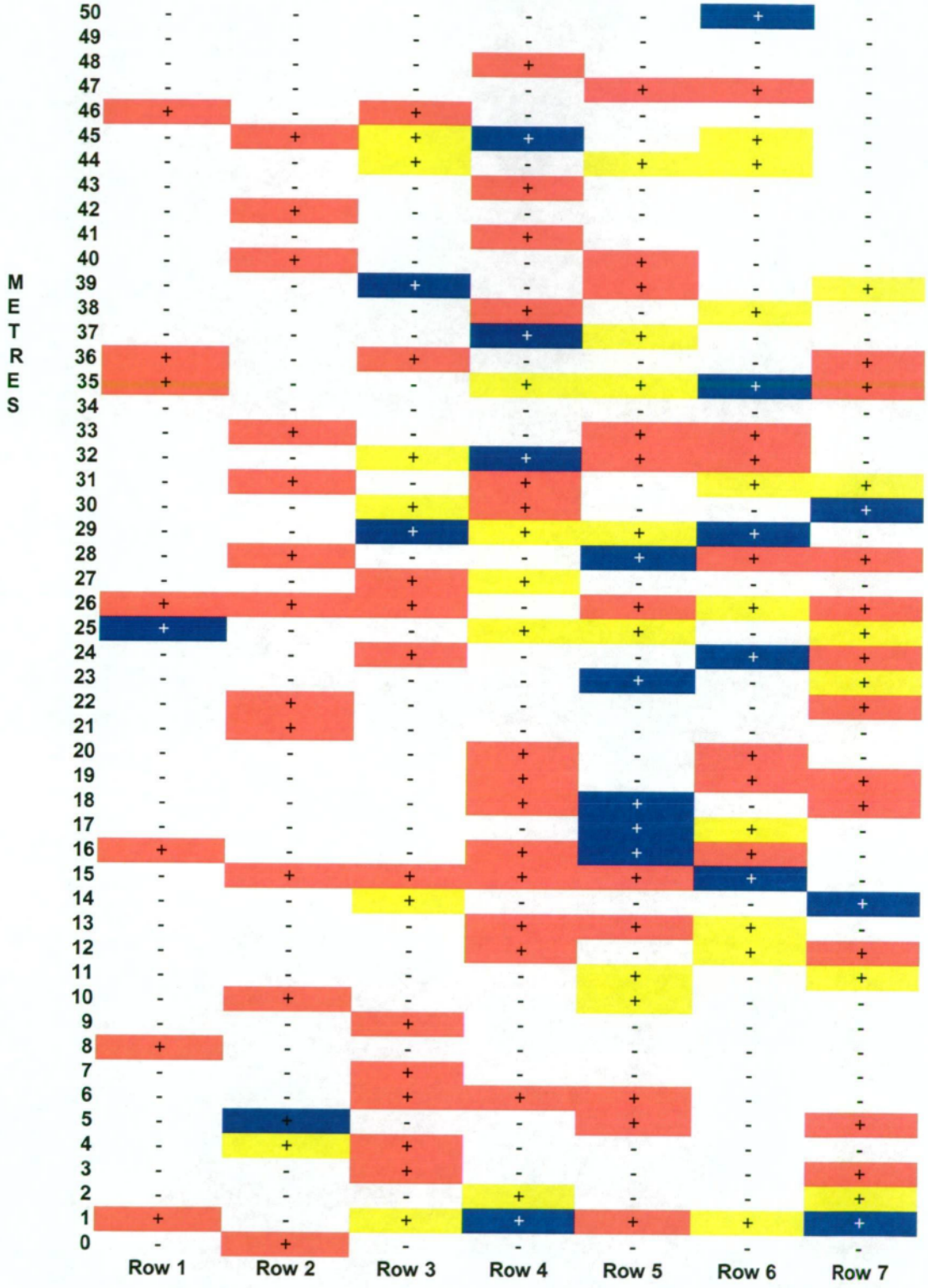
Appendix IV-1b. Map of PVS infection in transect trial at field 1 at 129 DAP.



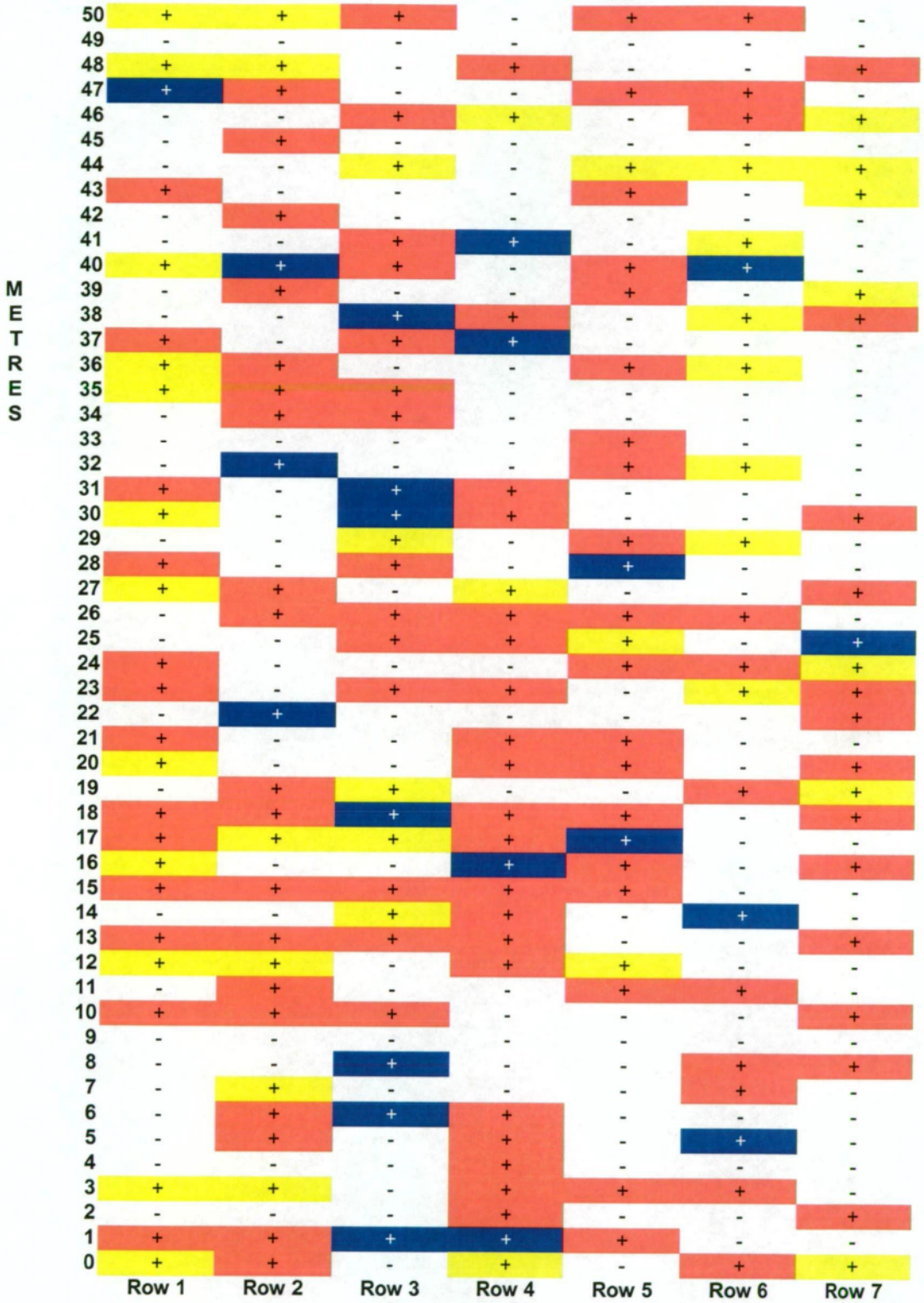
Appendix IV-2a. Map of PVS and PVX infection in transect trial at field 2 at 58 DAP.



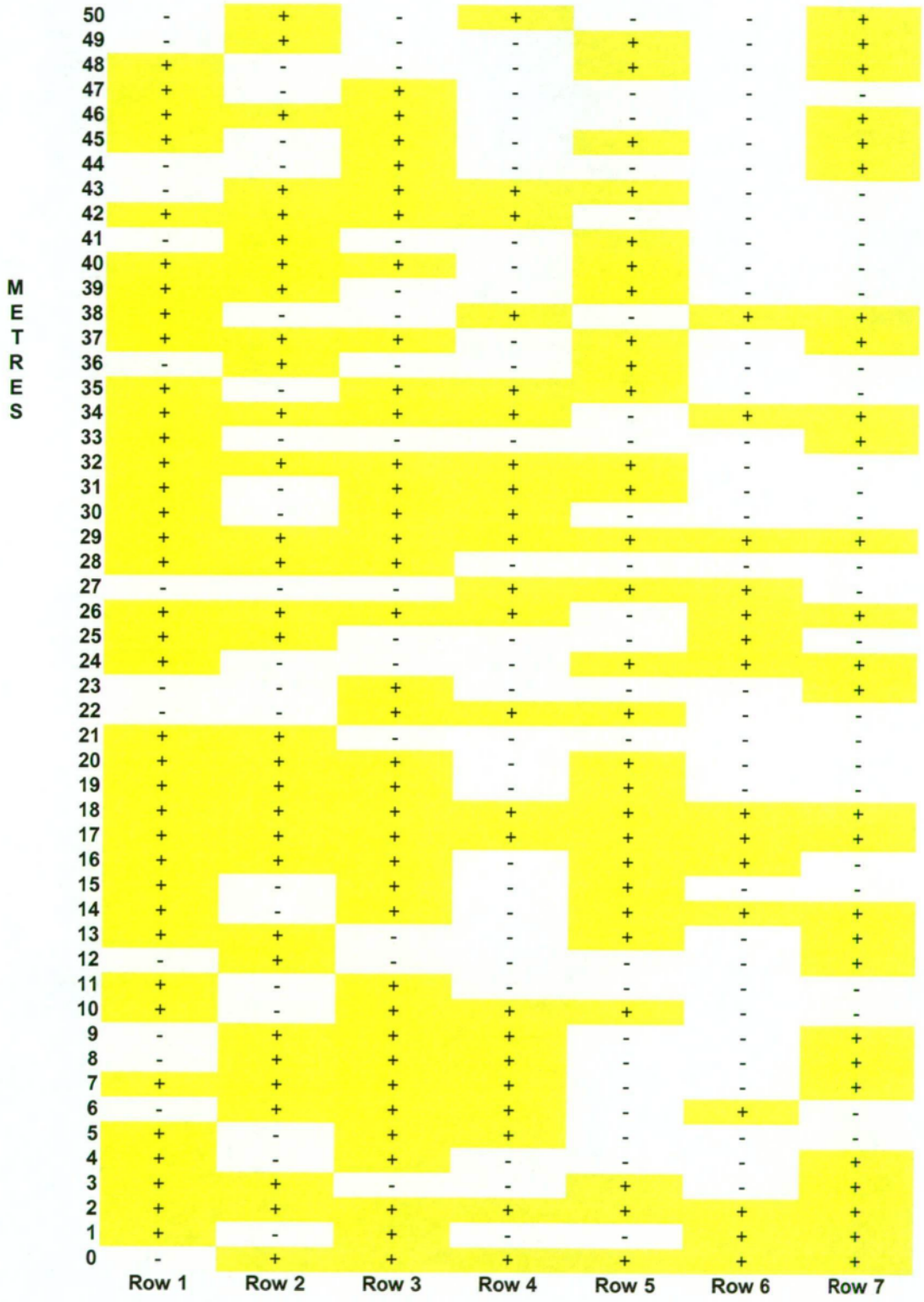
Appendix IV-2b. Map of PVS and PVX infection in transect trial at field 2 at 132 DAP.



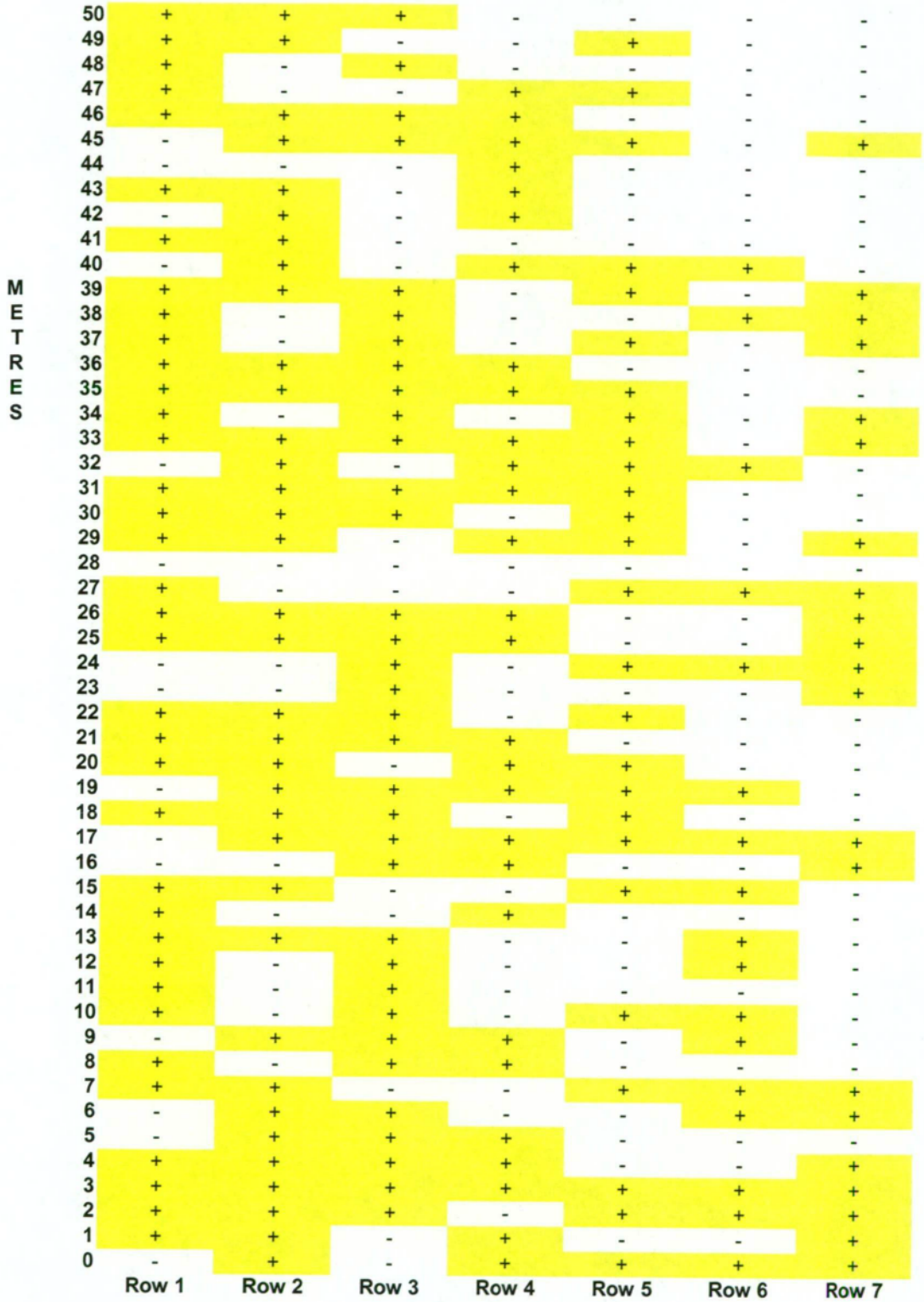
Appendix IV-3a. Map of PVS and PVX infection in transect trial at field 3 at 31 DAP.



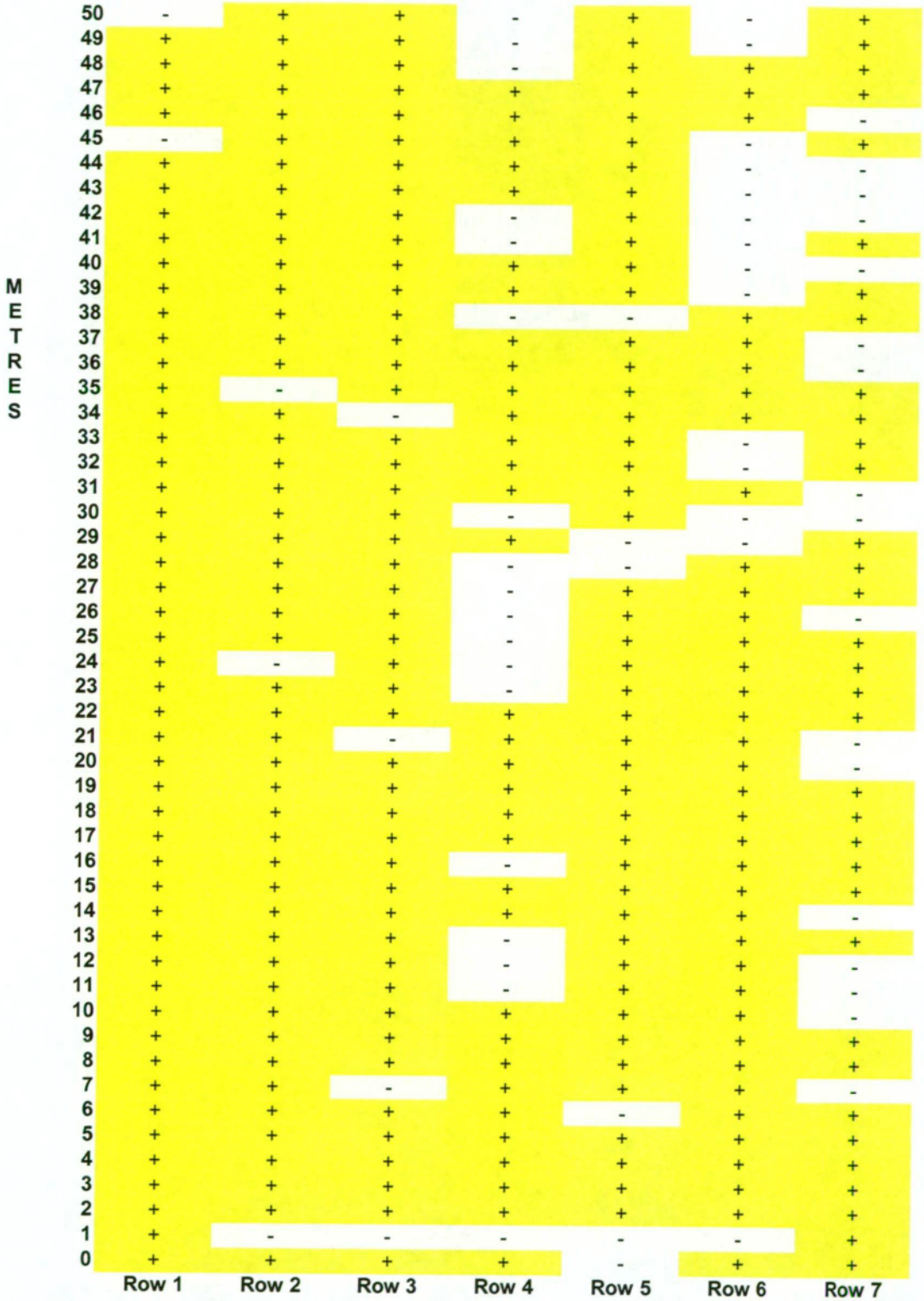
Appendix IV-3b. Map of PVS and PVX infection in transect trial at field 3 at 107 DAP.



Appendix IV.4a. Map of PVS and PVX infection in transect trial at field 4 at 30 DAP.



Appendix IV-4b. Map of PVS and PVX infection in transect trial at field 4 at 54 DAP.



Appendix IV-4c. Map of PVS and PVX infection in transect trial at field 4 at 105 DAP.

Appendix V Visual representation of PVS and PVX incidence in infected plants in plot trials

Appendices V-1. (a,b), V-2 (a,b), V-3 (a,b) and V-4 (a,b,c). A visual representation of *Potato virus S* (PVS) and *Potato virus X* (PVX) incidence in plot trials in seed potato (var. Russet Burnbank) in Tasmania at field 1 (31 and 107 days after planting, DAP), field 2 (56 and 132 DAP), field 3 (31 and 107 DAP) and field 4 (30, 54 and 105 DAP) are shown in Appendix V(1-4), respectively during 2003 and 2004 growing seasons.

Key for Appendices V-1 (a,b), V-2 (a,b), V-3 (a,b) and V-4 (a,b,c)

Healthy

PVS infection

PVX infection

7 PVS 35%	**14** PVS 60%	**21** PVS 45%	**28** PVS 40%	**35** PVS 50%	**42** PVS 55%	**49** PVS 35%
6 PVS 50%	**13** PVS 50%	**20** PVS 45%	**27** PVS 50%	**34** PVS 55%	**41** PVS 75%	**48** PVS 35%
5 PVS 55%	**12** PVS 65%	**19** PVS 40%	**26** PVS 70%	**33** PVS 55%	**40** PVS 60%	**47** PVS 80%
4 PVS 25%	**11** PVS 45%	**18** PVS 35%	**25** PVS 47%	**32** PVS 60%	**39** PVS 60%	**46** PVS 25%
3 PVS 40%	**10** PVS 30%	**17** PVS 70%	**24** PVS 50%	**31** PVS 45%	**38** PVS 45%	**45** PVS 50%
2 PVS 75%	**9** PVS 50%	**16** PVS 50%	**23** PVS 50%	**30** PVS 55%	**37** PVS 75%	**44** PVS 40%
1 PVS 30%	**8** PVS 45%	**15** PVS 55%	**22** PVS 35%	**29** PVS 70%	**36** PVS 55%	**43** PVS 45%

a)

7 PVS 30%	**14** PVS 25%	**21** PVS 35%	**28** PVS 35%	**35** PVS 55%	**42** PVS 20%	**49** PVS 65%
6 PVS 55%	**13** PVS 35%	**20** PVS 60%	**27** PVS 25%	**34** PVS 50%	**41** PVS 35%	**48** PVS 90%
5 PVS 35%	**12** PVS 25%	**19** PVS 30%	**26** PVS 50%	**33** PVS 75%	**40** PVS 55%	**47** PVS 55%
4 PVS 55%	**11** PVS 45%	**18** PVS 55%	**25** PVS 0%	**32** PVS 50%	**39** PVS 45%	**46** PVS 55%
3 PVS 25%	**10** PVS 35%	**17** PVS 40%	**24** PVS 55%	**31** PVS 45%	**38** PVS 65%	**45** PVS 80%
2 PVS 25%	**9** PVS 35%	**16** PVS 15%	**23** PVS 35%	**30** PVS 60%	**37** PVS 70%	**44** PVS 50%
1 PVS 40%	**8** PVS 20%	**15** PVS 35%	**22** PVS 60%	**29** PVS 45%	**36** PVS 25%	**43** PVS 75%

b)

Appendix V-1 (a,b) Virus incidence in plots from field 1 a) plants infected at 31 DAP, and b) before senescence at 107 DAP

7 PVS: 0% PVX: 0%	14 PVS: 0% PVX: 15%	21 PVS: 0% PVX: 0%	28 PVS: 0% PVX: 10%	35 PVS: 0% PVX: 0%	42 PVS: 0% PVX: 0%	49 PVS: 0% PVX: 5%
6 PVS: 0% PVX: 15%	13 PVS: 0% PVX: 15%	20 PVS: 0% PVX: 15%	27 PVS: 0% PVX: 5%	34 PVS: 0% PVX: 0%	41 PVS: 0% PVX: 5%	48 PVS: 0% PVX: 5%
5 PVS: 0% PVX: 15%	12 PVS: 0% PVX: 0%	19 PVS: 0% PVX: 5%	26 PVS: 0% PVX: 0%	33 PVS: 0% PVX: 0%	40 PVS: 0% PVX: 0%	47 PVS: 0% PVX: 5%
4 PVS: 0% PVX: 20%	11 PVS: 0% PVX: 5%	18 PVS: 0% PVX: 10%	25 PVS: 0% PVX: 0%	32 PVS: 0% PVX: 20%	39 PVS: 0% PVX: 5%	46 PVS: 0% PVX: 5%
3 PVS: 0% PVX: 0%	10 PVS: 0% PVX: 0%	17 PVS: 0% PVX: 5%	24 PVS: 5% PVX: 10%	31 PVS: 0% PVX: 5%	38 PVS: 0% PVX: 10%	45 PVS: 0% PVX: 10%
2 PVS: 0% PVX: 10%	9 PVS: 0% PVX: 0%	16 PVS: 0% PVX: 10%	23 PVS: 0% PVX: 5%	30 PVS: 0% PVX: 5%	37 PVS: 5% PVX: 5%	44 PVS: 5% PVX: 0%
1 PVS: 0% PVX: 0%	8 PVS: 5% PVX: 0%	15 PVS: 5% PVX: 5%	22 PVS: 0% PVX: 0%	29 PVS: 0% PVX: 5%	36 PVS: 0% PVX: 0%	43 PVS: 0% PVX: 0%

a)

7 PVS: 0% PVX: 0%	14 PVS: 0% PVX: 0%	21 PVS: 0% PVX: 0%	28 PVS: 0% PVX: 35%	35 PVS: 0% PVX: 20%	42 PVS: 0% PVX: 20%	49 PVS: 0% PVX: 70%
6 PVS: 0% PVX: 10%	13 PVS: 0% PVX: 25%	20 PVS: 0% PVX: 25%	27 PVS: 0% PVX: 5%	34 PVS: 0% PVX: 20%	41 PVS: 0% PVX: 15%	48 PVS: 0% PVX: 0%
5 PVS: 0% PVX: 30%	12 PVS: 10% PVX: 20%	19 PVS: 0% PVX: 0%	26 PVS: 0% PVX: 0%	33 PVS: 0% PVX: 30%	40 PVS: 0% PVX: 15%	47 PVS: 0% PVX: 10%
4 PVS: 0% PVX: 10%	11 PVS: 0% PVX: 40%	18 PVS: 0% PVX: 15%	25 PVS: 0% PVX: 20%	32 PVS: 0% PVX: 50%	39 PVS: 0% PVX: 30%	46 PVS: 0% PVX: 20%
3 PVS: 0% PVX: 0%	10 PVS: 0% PVX: 5%	17 PVS: 0% PVX: 5%	24 PVS: 0% PVX: 0%	31 PVS: 0% PVX: 5%	38 PVS: 0% PVX: 30%	45 PVS: 0% PVX: 20%
2 PVS: 0% PVX: 15%	9 PVS: 0% PVX: 0%	16 PVS: 15% PVX: 0%	23 PVS: 0% PVX: 25%	30 PVS: 0% PVX: 0%	37 PVS: 0% PVX: 0%	44 PVS: 0% PVX: 0%
1 PVS: 0% PVX: 0%	8 PVS: 0% PVX: 0%	15 PVS: 0% PVX: 5%	22 PVS: 7% PVX: 35%	29 PVS: 0% PVX: 30%	36 PVS: 0% PVX: 0%	43 PVS: 0% PVX: 45%

b)

Appendix V-2 (a,b) Virus incidence of PVS and PVX in plots from field 2, a) plants infected at 56 DAP, and b) before senescence at 132 DAP.

7 PVS: 20% PVX: 0%	14 PVS: 10% PVX: 0%	21 PVS: 5% PVX: 0%	28 PVS: 0% PVX: 0%	35 PVS: 15% PVX: 0%	42 PVS: 10% PVX: 0%	49 PVS: 5% PVX: 0%
6 PVS: 15% PVX: 15%	13 PVS: 10% PVX: 0%	20 PVS: 15% PVX: 0%	27 PVS: 20% PVX: 0%	34 PVS: 10% PVX: 0%	41 PVS: 30% PVX: 0%	48 PVS: 0% PVX: 0%
5 PVS: 10% PVX: 15%	12 PVS: 15% PVX: 0%	19 PVS: 25% PVX: 0%	26 PVS: 15% PVX: 0%	33 PVS: 10% PVX: 10%	40 PVS: 5% PVX: 5%	47 PVS: 5% PVX: 0%
4 PVS: 10% PVX: 5%	11 PVS: 20% PVX: 0%	18 PVS: 15% PVX: 10%	25 PVS: 10% PVX: 0%	32 PVS: 20% PVX: 15%	39 PVS: 5% PVX: 0%	46 PVS: 10% PVX: 0%
3 PVS: 5% PVX: 10%	10 PVS: 10% PVX: 0%	17 PVS: 0% PVX: 15%	24 PVS: 15% PVX: 0%	31 PVS: 10% PVX: 0%	38 PVS: 10% PVX: 0%	45 PVS: 10% PVX: 0%
2 PVS: 10% PVX: 10%	9 PVS: 10% PVX: 0%	16 PVS: 10% PVX: 0%	23 PVS: 20% PVX: 0%	30 PVS: 0% PVX: 0%	37 PVS: 20% PVX: 0%	44 PVS: 20% PVX: 5%
1 PVS: 10% PVX: 5%	8 PVS: 10% PVX: 0%	15 PVS: 10% PVX: 5%	22 PVS: 0% PVX: 0%	29 PVS: 0% PVX: 5%	36 PVS: 10% PVX: 0%	43 PVS: 10% PVX: 0%

a)

7 PVS: 20% PVX: 15%	14 PVS: 55% PVX: 0%	21 PVS: 45% PVX: 0%	28 PVS: 15% PVX: 0%	35 PVS: 0% PVX: 0%	42 PVS: 15% PVX: 0%	49 PVS: 10% PVX: 0%
6 PVS: 0% PVX: 10%	13 PVS: 10% PVX: 0%	20 PVS: 5% PVX: 0%	27 PVS: 30% PVX: 0%	34 PVS: 30% PVX: 5%	41 PVS: 15% PVX: 0%	48 PVS: 10% PVX: 5%
5 PVS: 5% PVX: 0%	12 PVS: 35% PVX: 15%	19 PVS: 5% PVX: 0%	26 PVS: 10% PVX: 0%	33 PVS: 20% PVX: 0%	40 PVS: 0% PVX: 5%	47 PVS: 5% PVX: 5%
4 PVS: 10% PVX: 0%	11 PVS: 5% PVX: 10%	18 PVS: 15% PVX: 10%	25 PVS: 5% PVX: 0%	32 PVS: 30% PVX: 0%	39 PVS: 15% PVX: 5%	46 PVS: 10% PVX: 0%
3 PVS: 25% PVX: 10%	10 PVS: 5% PVX: 0%	17 PVS: 15% PVX: 10%	24 PVS: 10% PVX: 0%	31 PVS: 25% PVX: 5%	38 PVS: 5% PVX: 20%	45 PVS: 20% PVX: 10%
2 PVS: 20% PVX: 0%	9 PVS: 20% PVX: 0%	16 PVS: 20% PVX: 5%	23 PVS: 10% PVX: 0%	30 PVS: 30% PVX: 10%	37 PVS: 5% PVX: 10%	44 PVS: 5% PVX: 0%
1 PVS: 20% PVX: 5%	8 PVS: 10% PVX: 25%	15 PVS: 30% PVX: 0%	22 PVS: 10% PVX: 0%	29 PVS: 35% PVX: 0%	36 PVS: 10% PVX: 5%	43 PVS: 5% PVX: 0%

b)

Appendix V-3 (a,b) Virus incidence of PVS and PVX in plots from field 3, a) plants infected at 31 DAP, and b) before senescence at 107 DAP.

7 PVS 15%	**14** PVS 10%	**21** PVS 5%	**28** PVS 15%	**35** PVS 15%	**42** PVS 10%	**49** PVS 50%
6 PVS 20%	**13** PVS 0%	**20** PVS 25%	**27** PVS 30%	**34** PVS 25%	**41** PVS 35%	**48** PVS 50%
5 PVS 15%	**12** PVS 60%	**19** PVS 30%	**26** PVS 5%	**33** PVS 20%	**40** PVS 15%	**47** PVS 60%
4 PVS 5%	**11** PVS 35%	**18** PVS 0%	**25** PVS 30%	**32** PVS 10%	**39** PVS 40%	**46** PVS 40%
3 PVS 20%	**10** PVS 40%	**17** PVS 5%	**24** PVS 20%	**31** PVS 25%	**38** PVS 55%	**45** PVS 40%
2 PVS 15%	**9** PVS 10%	**16** PVS 15%	**23** PVS 0%	**30** PVS 35%	**37** PVS 50%	**44** PVS 60%
1 PVS 35%	**8** PVS 20%	**15** PVS 40%	**22** PVS 15%	**29** PVS 20%	**36** PVS 40%	**43** PVS 5%

c)

Appendix V-4 (a,b,c) cont. Virus incidence of PVS and PVX in plot trials from field 4, a) at immergence at 30 DAP, b) before row closure at 54 DAP, and c) before senescence at 105 DAP.

7	14	21	28	35	42	49
PVS 15%	PVS 15%	PVS 30%	PVS 15%	PVS 45%	PVS 55%	PVS 40%
6	13	20	27	34	41	48
PVS 30%	PVS 15%	PVS 20%	PVS 25%	PVS 35%	PVS 25%	PVS 45%
5	12	19	26	33	40	47
PVS 15%	PVS 20%	PVS 20%	PVS 30%	PVS 25%	PVS 35%	PVS 35%
4	11	18	25	32	39	46
PVS 10%	PVS 30%	PVS 10%	PVS 15%	PVS 40%	PVS 25%	PVS 40%
3	10	17	24	31	38	45
PVS 15%	PVS 35%	PVS 10%	PVS 25%	PVS 35%	PVS 20%	PVS 25%
2	9	16	23	30	37	44
PVS 15%	PVS 30%	PVS 30%	PVS 25%	PVS 35%	PVS 15%	PVS 50%
1	8	15	22	29	36	43
PVS 25%	PVS 15%	PVS 15%	PVS 20%	PVS 15%	PVS 30%	PVS 50%

a)

7	14	21	28	35	42	49
PVS 25%	PVS 5%	PVS 25%	PVS 25%	PVS 15%	PVS 35%	PVS 30%
6	13	20	27	34	41	48
PVS 15%	PVS 5%	PVS 25%	PVS 5%	PVS 34%	PVS 35%	PVS 40%
5	12	19	26	33	40	47
PVS 15%	PVS 40%	PVS 25%	PVS 10%	PVS 15%	PVS 25%	PVS 20%
4	11	18	25	32	39	46
PVS 15%	PVS 20%	PVS 15%	PVS 5%	PVS 20%	PVS 15%	PVS 40%
3	10	17	24	31	38	45
PVS 20%	PVS 10%	PVS 15%	PVS 10%	PVS 20%	PVS 10%	PVS 30%
2	9	16	23	30	37	44
PVS 15%	PVS 20%	PVS 20%	PVS 15%	PVS 25%	PVS 35%	PVS 45%
1	8	15	22	29	36	43
PVS 15%	PVS 15%	PVS 25%	PVS 15%	PVS 15%	PVS 30%	PVS 30%

b)

Appendix V-4 (a,b,c) cont. Virus incidence of PVS and PVX in plot trials from field 4, a) at immergence at 30 DAP, and b) before row closure at 54 DAP.